AD-A260 006





CONTRACT NO.: DAMD17-88-Z-8023

TITLE: EFFECT OF FOOD, DIET AND NUTRITION ON MILITARY READINESS AND PREPAREDNESS OF ARMY PERSONNEL AND DEPENDENTS IN A PEACETIME ENVIRONMENT

PRINCIPAL INVESTIGATOR: Donna H. Ryan

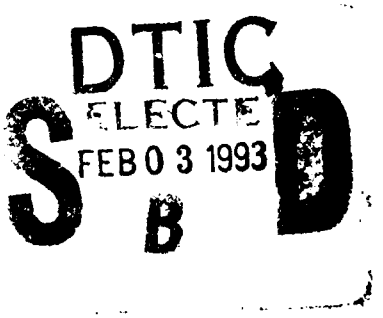
PI ADDRESS: Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, Louisiana 70808-4124

REPORT DATE: August 15, 1992

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited



93-01908



SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Pennington Biomedical Research Center 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 6400 Perkins Road Baton Rouge, LA 70808-4124	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD 17-88-Z-8023
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
63002A	3M2-63002D819	AI	150

11. TITLE (Include Security Classification)
Effect of Food, Diet and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment

12. PERSONAL AUTHOR(S)
Donna H. Ryan, M.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 7/28/88 TO 7/27/92	1992 August 15	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	RA 3, Nutrition, Health Promotion, Military Readiness, Nutritional Status

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Five projects underway at the Pennington Biomedical Research Center (PBRC) are reported herein. The Support Laboratory for Human and Food Samples supports U.S. Army Research Institue of Environmental Medicine (USARIEM) field reseach in sites ranging from Alaska to Bolivia. The Stable Isotope Laboratory supports USARIEM research by determining energy expenditure in the field. The Fort Polk Heart Smart Project has completed an assessment of nutritional and exercise habits of military wives, a project that evaluates screening for cardiovascular risk factors and a project that assesses a health promotion model in military families. The Nutrition and Military Performance Laboratory conducted basic research in the effect of diet on behavior through biochemical, physiologic, and behavioral assessment studies. The Menu Modification Project has analyzed and altered two sets of Army menus.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b. TELEPHONE (Include Area Code) 301-619-7321 / 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

PI Signature: Donna H Regan Date: 8/26/92

DTIC QUALITY INSPECTED 3

Accession For
NTIS CRA&I ☑
DTIC TAB ☐
Unannounced ☐
Justification
By
Distribution/
Availability Codes
Dist | Avail and/or Special
A-1

TABLE OF CONTENTS

Introduction 1

Project #1. Clinical Research Laboratory 3

Project #2. Stable Isotope Laboratory. 11

Project #3. Nutrition and Military Performance Laboratory. . . .19

Project #4. Fort Polk Heart Smart Project. 47

Project #5. Menu Modification Study. 53

Appendices 59

FINAL REPORT
U.S. ARMY GRANT
7/27/88 - 7/28/92

Background and Overview

On July 27, 1988, the U.S. Army awarded grant #DAMD-17-88-Z-8023, "The Effect of Food, Diet and Nutrition on Military Readiness and Preparedness of Military Personnel and Dependents in a Peace Time Environment" to the Pennington Center. The total grant award was $3,500,000. The U.S. Army staff at the U.S. Army Institute of Environmental Medicine (USARIEM) reviewed the projects at a site visit to the Pennington Center in August, 1988. The Committee on Military Nutrition Research reviewed and approved research projects proposed by the Pennington Center at a site visit in 1988. The grant did not allow for equipment purchase and the Pennington Center used other funding sources to provide over $1.5 million equipment to support these research projects.

The results of this research have been completed on or ahead of schedule with high quality and are detailed in four annual and 16 quarterly reports.

The Pennington Center developed four projects to meet U.S. Army objectives published in the Grant. They are detailed on the following page and listed below:

.Stable Isotope Laboratory for Determination of Energy Expenditure

.Support Laboratory for Human and Food Samples

.Menu Modification Project

.Fort Polk Heart Smart Project

In addition, Dr. Chandan Prasad developed a project with U.S. Army approval to study the effect of stressful situations and inadequate nutritional intake on performance. This project assembled a basic science team including senior investigator, neurophysiologist, neuroanatomist, behavioral assessment scientist, and neurobiochemist. This project is the **"Nutrition and Military Performance Laboratory."**

One of the research projects, the Fort Polk Heart Smart Project, terminated on schedule on July 28, 1992. Grant funds enabled the continuation of four of the projects initiated in conjunction with USARIEM. Those projects are the Stable Isotope Laboratory, the Support Laboratory for Human and Food Samples Nutrition Services, the Nutrition and Military Performance Laboratory, and the Menu Modification Project until July 28, 1992

Listed below are the Grant objectives and the projects developed by the Pennington Center that fulfilled those objectives.

U.S. ARMY GRANT DAMD 17-88-Z-8023

Army Objectives Objectives	PBRC Projects to Meet
1. "Establish a Nutritional Health promotion Research Development Test and Evaluation (RDTE) Center for military personnel and dependents in a peacetime environment to accomplish the following: a. assess the nutritional adequacy of the diet of military personnel to promote health and military readiness; b. evaluate and develop military dietary programs for dining facilities, commissaries and other food service facilities operated by the military c. monitor the nutritional status of military personnel and their family members; and d. develop and evaluate military nutrition, education, and health promotion programs.	1. The Pennington Center established the following two projects to fulfill research objective one: *Fort Polk Heart Smart Project: Dr. David Harsha and Dr. Gerald Berenson produced a three phase project at Fort Polk, Louisiana. In phase one, 200 military personnel and dependents were evaluated for dietary intake and physical activity. In phase two, 140 military families were screened for cardiovascular disease risk factors. In phase three, a model health promotion program lasting 12 weeks was implemented and evaluated for 60 families. *Menu Modification Project: Dr. Nena Cross analyzed and modified 10 garrison menus in phase one. In phase two she refined 10 additional menus to meet revised nutritional guidelines and tested the menus in the garrison setting.
2. Provide nutrition laboratory research support to the army's military nutrition research program at USARIEM to accomplish the following: a. provide biochemical assessment of nutrition status; b. perform food biochemistry analysis; and c. establish and perform stable isotope methodologies for assessment."	2. The three components of objective two were met by the following: *Support Laboratory for human and food samples. In the last fiscal year the laboratory performed over 15,000 tests for the Army. *Stable Isotope Laboratory performs heavy water ($^{2}H_{2}{}^{18}O$) determination of energy expenditure, the only technique that allows caloric expenditure measurement in the field. Studies of U.S. soldiers were done in Alaska and Bolivia.

We are indebted to the following army investigators for their assistance in this work: Colonel E. Wayne Askew, Colonel David S. Schnakenberg, Colonel Dale Block, Major Judy Turcotte, Colonel Garland McCarty, Colonel Fred Cecere, Ms. Doris Sherman, Colonel Denniston, Major Henley, Major Mary Mays, Dr. Harris Lieberman, and Major Cecelia Thomas.

We were also assisted in the development of U.S. Army projects by former Acting Director of Pennington Biomedical Research Center, William Pryor, and Executive Director, George Bray. The support of faculty and leadership of the LSU A & M College and the LSU Medical Center, under the leadership of LSU System President, Dr. Allen Copping, was instrumental in developing and executing these projects.

Discussions of individual projects funded under this grant follow.

I. Clinical Research Laboratory

Introduction

One of the research objective of the Army grant is to "provide nutrition laboratory research support to the army's military nutrition research program at USARIEM to accomplish the following:

a. provide biochemical assessment of nutrition status;

b. perform food biochemistry analysis..."

The clinical research laboratory at Pennington Biomedical Research Center has dedicated itself to fulfilling these objectives. The laboratory has been equipped, employees hired, methods of analysis developed, and analyses performed for the research program at USARIEM. The laboratory now supports clinical research projects in addtiion to USARIEM research and now serves NIH, USDA, AHA and privately funded research in addition to Department of Defense-funded research.

Progress

1. Progress on Equipment

Equipment purchased and performing analyses during the period of this grant include the following:

a. Beckman Synchron CX5 automated clinical chemistry system;

b. Coulter STKS automated hematology system with five part differential;

c. Perkin Elmer P1000 ICP emission spectrometer;

d. Perkin Elmer Z5100 graphite furnace atomic absorption spectrophotometer with Zeeman background correction;

e. Antek Nitrogen analyzer;

f. Two Hewlett Packard 1090M HPLC systems with diode array and fluorescent detectors and autosamplers;

g. Bio Rad HPLC system with electrochemical detector for catecholamine analysis;

h. Clinitek 2000 automated urine dip stick reader;

i. Packard RIASTAR 20 well gamma counter;

j. Hewlett Packard UV-Vis diode array spectrophotometer;

k. Hewlett Packard GC with IR detector;

l. CEM microwave digestion system;

m. Dionex computerized system for integration and controlling the nitrogen and catecholamine analyzers.

2. Progress on Method Development

a. General Chemistry

Most routine chemistry analyses are performed on the Beckman Synchron CX5 automated chemistry system. A description of this system is given in the Second Annual Report, pg 8 (1). Tests which we are currently operational on this system include:

glucose	albumin (BCP-humans)
urea	calcium
creatinine	phosphorus
sodium	magnesium
potassium	aspartate transaminase
chloride	alkanine transaminase
carbon dioxide	alkaline phosphatase
uric acid	creatine kinase
total protein	lactate dehydrogenase
amylase	GGT
total bilirubin	direct bilirubin
cholesterol	HDL cholesterol
triglyceride	iron
iron binding capacity	albumin (BCG-animals)
free fatty acids	glycerol
beta hydroxybutyrate	lactic acid
ammonia	alcohol
para aminobenzoic acid	vitamin C
lipase	red cell AST
red cell glutathione reductase	red cell transketolase

Tests which were developed at PBRC include free fatty acids, glycerol, beta hydroxybutyrate, lactic acid, ammonia, para aminobenzoic acid, vitamin C, alcohol, lipase, and the red cell enzymes AST, glutathione reductase, and transketolase, as well as the in vitro stimulations by their respective cofactors. Information on the details of methods for free fatty acids, glycerol, beta hydroxybutyrate, lactic acid, and ammonia were given in the 1990 Annual Report, Tables 1-3 (1). Recovery was nearly quantitative in all cases. These methods have been in use for nearly three years. Correlation studies are presented in the appendix.

Four HDL cholesterol methods have been evaluated (heparin/Mn, isoelectric point phosphotungstic acid, Beckman phosphotungstic acid, and 50,000 molecular weight dextran sulfate. The heparin method was found to be unacceptable for use with the enzymatic cholesterol method on the CX5. The other three methods were correlated as shown in the appendix and Section I of the Third Annual Report (2). As suggested by Major Friedl we have switched over to the dextran sulfate method for this analysis.

Para-aminobenzoic acid analysis has been automated on the CX5 for the purpose of tracking food intake. Linearity, precision and recovery are good.

Vitamin C assay was set-up on the CX5. See the Fourth Annual Report for details (3).

b. Nitrogen Analysis

Total urinary, fecal, sweat, water, or food nitrogen are measured on our model 703C pyrochemiluminescent nitrogen system (Antek Instruments, Inc., Houston, TX 77076). Urine samples are diluted approximately 1:121 prior to analysis. To analyze food or feces, a homogenate (0.2-1 g) is digested with 10 ml sulfuric acid, 1 ml hydrogen peroxide, and 1 ml of 10% cupric sulfate. The digest is diluted to 200 ml. Linearity , precision, and accuracy by recovery studies were determined for this method (Section I and the appendix of the Third Annual Report) (2).

c. Minerals and Trace Elements

Sweat, urine, water, food, and feces are analyzed on the Perkin Elmer P1000 ICP emission spectrometer. Sweat, urine, and water are analyzed directly or diluted with water. Feces and food are analyzed following homogenization and digestion with 10 ml of sulfuric acid, 1 ml of hydrogen peroxide, and 1 ml of 10% cupric sulfate. The digest is then diluted with water. It was found that when calibration was performed using aqueous standards in 0.1 mol/L nitric acid that there was a significant matrix effect due to the digestion solution. To counteract this standards were prepared in the digestion matrix. Results for linearity, precision, and

recovery using these standards were given in Table 1 and the appendix of the Third Annual Report (2).

Trace element analyses have been set up for platinum and gallium by graphite furnace atomic absorption spectrophotometry.

d. Coulter STKS

The Coulter STKS is now routinely used in the clinical laboratory. A study was performed comparing the correlation of the five part differential with that obtained manually. These results are shown in the appendix. QC results compare very well to our peers and we have done very well in the CAP survey (appendix of the Third Annual Report) (2).

e. HPLC Methods

Work on amino acid separation by HPLC has been ongoing during the last two years of this grant (1). A very good separation was achieved; however, co-elution of GABA and arginine has caused problems. Combining several methods, we have what we believe to be a very good analysis of amino acids. The method has yet to be investigated to determine recovery, precision, and linearity. Work on this method will continue funded from other sources until satisfactory results are obtained.

A method for the analysis of caffeine was set-up by reverse phase HPLC. Verification of this method by assayed controls was performed.

We developed a method of analysis for Vitamin A by reverse phase HPLC. The conditions thus far have consisted of using a mobile phase of 100% methanol through a C18 column. We have successfully separated retinol, retinyl palmitate as of this time. Recovery is nearly quantitative when the assay is performed using external standardization rather than an internal standardization. The recovery of the internal standard is variable for obscure reasons, resulting in non reproducible results. This method also has the capability of separating Vitamin E (alpha tocopferol). To date, however, we have not verified the precision, linearity, and recovery for this.

f. Radioimmunoassays

RIA's which were set-up include ferritin, Vitamin B12, red cell and serum folate, insulin, vasopressin, aldosterone, growth hormone, prolactin, 25 hydroxy vitamin D, and cortisol. These methods have all been evaluated by running assayed controls, performing routine quality control, and running samples from an interlaboratory survey program for hormones.

g. Catecholamines

Catecholamine analysis has been set up and these are being performed routinely on plasma and urine samples for studies being conducted at PBRC. See the Fourth Annual Report for details (3).

3. Progress on Quality Control

Procedure manuals for Chemistry, Urinalysis, Immunoassay, Quality Control, and Policies have been written and these policies and protocols put into practice. These are available for review at the Pennington Center. Quality control practices include routine monitoring of refrigerator and freezer temperatures, water quality, and reagent receipt and acceptability. Biannual checks of the linearity of each method, precision and accuracy of pipets, centrifuge speed and temperatures have been instituted (see appendix for examples of these protocols).

Routine quality control has been ongoing in the chemistry, hematology, immunoassay, and urinalysis sections. The chemistry and hematology internal QC results are compared monthly with other users of the same lot numbers across the country. We have generally rated very well on these reports. Examples of monthly reports for chemistry and hematology are included in the appendix of the Third Annual Report (2) and Fourth Annual Report (3).

We have been subscribing to the College of American Pathologist (CAP) external lab survey, as well as the Endo Survey of the American Association of Clinical Chemists since January, 1991. We have not reported results for the Endo survey because of the non-routine nature of our performance of immunoassays. We have, however, run these samples within our lab setting and compared results to the survey respondents and done very well. CAP survey results have been very favorable. Copies of these are also included in the appendix of the Third Annual Report (2) and Fourth Annual Report (3).

MAJOR SCIENTIFIC ACHIEVEMENTS

The major scientific achievement of the Support Laboratory for Human and Food Samples has been in aiding progress on U.S. Army research projects.

The following studies have been completed for USARIEM:

Carbohydrate Load Study

A total of 51 samples were analyzed for ammonia, B-hydroxybutyrate, glucose, glycerol, lactate, non esterified fatty acids, and triglyceride. In addition, 180 samples were analyzed for plasma lactate. In total, 557 tests were performed. The results for this study are shown in the Second Annual Report (1).

Alaska Winter Field Feeding Evaluation (1990)

A total of 156 samples were analyzed for Chemistry 22 panels plus HDL cholesterol. In total, 3588 tests were performed.

West Point Nutritional Assessment Study

Approximately 400 samples were obtained for the analysis of serum lipids, iron, TIBC, ferritin, Vitamin B_{12}, and folic acid. Also, 94 samples were analyzed for red blood cell folic acid. In all, 1645 analyses were performed. The report is shown in the appendix of the Second Annual Report (1).

Sodium Depletion Study

The sodium depletion study was completed this year. Nitrogen, sodium, potassium, calcium, magnesium, and phosphorus were measured in urine, sweat, water, feces, and food. Due to the discovery of a matrix interference, the analyses were repeated a second time for the minerals on the food and feces. These were done using standards containing the digestion matrix (sulfuric acid/hydrogen peroxide/cupric acetate). Captain Moore indicated to us that the results for the MRE samples were too low for sodium. He asked that we investigate the problem. Half of the original MRE samples were repeated using a different digestion mixture (nitric acid/hydrogen peroxide). In the meantime, it was discovered that a mathematical error had been made in analysis of some of the food samples. Some foods had been homogenized with an equal weight of water; this water had not been taken into account in the final calculations. Doing so doubled all of the mineral weights per gram of food and total concentration of each mineral in some of the samples. All of the MRE samples and some of the other foods were processed this way. An amended report was prepared. The second set of digested MRE's matched the corrected concentrations of mineral from the first digestion very well. In addition, a second set of MRE foods were received from Natick in order for us to check our methods. We digested these in the same manner as the first set (sulfuric acid/hydrogen peroxide/cupric sulfate). The results for MRE foods which were the same as the first shipment agreed very favorably with the first batch (2). A total of 637 samples (urine, water, sweat, feces, and food) were analyzed for sodium, potassium, calcium, magnesium, phosphorus, and nitrogen for a grand total of 3822 tests (not including the reruns).

Alaska 91

Serum samples from the Alaska 91 study were received and 61 samples were processed for glucose, BUN, creatinine, total protein, cholesterol, triglyceride, HDL, lactate, non esterified fatty acids, beta hydroxybutyrate, and glycerol in serum and creatinine and nitrogen in urine. The report is shown in the appendix of the

Third Annual Report (2). A total of 61 samples were analyzed for a grand total of 500 tests.

Pikes Peak

Urine samples from the Pikes Peak study were received this year and processed for urinary nitrogen and creatinine. The report is shown in the appendix of the Third Annual Report (2). A total of 177 tests were performed on 97 samples.

Bolivia High Altitude Study

A total of 116 urine samples were analyzed for nitrogen and creatinine in this study (232 tests).

Survival Study

Serum samples from the Survival Study were received and analyzed for a chemistry panel, glycerol, lactic acid, ferritin, TIBC, and HDL cholesterol. Most samples were repeated at least once due to the very abnormal results found. These results were confirmed in most cases. 60 samples were analyzed for a total of 1500 tests.

Ranger Study

The first shipment from the Ranger study was received in July 1991. This was followed by four more shipments. These samples were analyzed for a chemistry panel and HDL cholesterol, beta hydroxybutyrate, glycerol, lactate, free fatty acids, iron, TIBC, ferritin, 25 hydroxy vitamin D, serum folate, RBC folate, vitamins A, C, and 25 hydroxy vitamin D, red cell AST, transketolase, and glutathione reductase with in vitro stimulation by pyridoxal phosphate, thiamine pyrophosphate, and FAD. A total of 10,642 tests were run (results in Quarterly Report 1/31/92 (4)).

MRE Study

This study included samples which were run for chem panel, glycerol, free fatty acids, lactate, HDL, beta hydroxybutyrate, serum vitamin B12, folate, 25 hydroxy vitamin D, RBC folate, RBC transketolase with in vitro stimulation by thiamine pyrophosphate, RBC glutathione reductase with in vitro stimulation by FAD, and RBC AST with in vitro stimulation by pyridoxal phosphate. A total of 7479 tests were performed.

Ranger 1.5 Study

This was a follow-up on eight of the GIs going through the Ranger study. The same tests were run as in the original Ranger Study. 312 tests were run (Quarterly Report 8/92 (5)).

Summary

Table 1 tabulates the studies and number of tests performed in each study.

TABLE 1. Studies Performed and/or Received by the Clinical Research Laboratory at PBRC.

Study	No. Samples	No. Tests	Completed
Carbohydrate Load	231	551	Jan 1990
Alaska 1990	156	3588	Mar 1990
West Point	494	1645	Jul 1990
Bolivia High Alt.	116	232	Oct 1990
Alaska 1991	61	500	Apr 1991
Pikes Peak	97	177	May 1991
Sodium Depletion	637	3900	Aug 1991
Survival Study	60	1500	Aug 1991
Ranger Study	1000	10642	Dec 1992
MRE Study	231	7479	Jun 1992
Ranger 1.5 Study	8	312	Jun 1992
TOTAL	**3091**	**30526**	

References

1. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Second Annual Report (8/1/89-7/31/90), August 15, 1990.

2. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Third Annual Report (7/29/90-7/28/91), 1991.

3. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Fourth Annual Report (7/29/91-7/28/92), 1992.

4. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Quarterly Report (1/31/92), 1992.

5. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Quarterly Report (8/92), 1992.

II. Stable Isotope Laboratory

Introduction

Establishment of a Stable Isotope Laboratory to support the Army's military nutrition research program at USARIEM was a research objective of US Army grant DAMD 17-88-G-8023. The Stable Isotope Laboratory at Pennington Biomedical Research Center/LSU was established in September, 1989 with the employment of James P. DeLany, Ph.D., as manager of the laboratory. A Finnigan Delta S Isotope Ratio Mass Spectrometer, a water-CO_2 equilibrator, a Breath Carousel for CO_2 Analysis, a Gas Chromatograph/Combustion Interface and a Multiport automatic tube cracker were purchased using USDA funds. The instrument was installed and calibrated, and the first Army samples analyzed by April, 1990. A Research Associate, Stable Isotope position was filled by Teodora Aranas, who began May 14, 1990. A second mass spectrometer (Finnigan MAT 252) has been installed, and a second Research Associate, Stable Isotope position has been filled by Julie Kumler.

The research conducted by the Stable Isotope Laboratory has been in the area of energy and water requirements of soldiers under harsh environmental conditions. The conditions studied have been in an arctic climate (2 studies), at altitude, during survival training and during Ranger Training. The method used to determine energy requirements was the doubly labeled water technique for measurement of energy expenditure.

The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals (1). The method is based on the premise that after a loading dose of $^2H_2{}^{18}O$, ^{18}O is eliminated as CO_2 and water, while deuterium is eliminated from the body as water. The rate of CO_2 production, and, hence, energy expenditure, can be calculated from the difference of the two elimination rates. Doubly labeled water, using the two-point method, is an ideal method for use in free-living subjects because it is noninvasive and nonrestrictive. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^2H_2{}^{18}O$, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries. Although these characteristics have been taken advantage of by zoologists for 20 years, doubly labeled water has only recently been applied to determination of energy expenditure in free-living human subjects (2-4)

The doubly labeled water method has been extensively validated in humans under controlled settings (5), but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations (2). Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Doubly Labeled Water Method

Total body water is calculated using ^{18}O isotopic enrichments measured predose, and 3 and 4 hours after the dose as follows:

$$TBW = (A/MW_d)(APE_d/100)18.02[1/R_{std}(E_s-E_p)](1/1.01)$$

where A is the dose given in grams, MW_d is the molecular weight of the dose water, APE_d is the atom percent excess enrichment of the dose water, R_{std} is the ratio of heavy to light isotope of SMOW, i.e., 2.005×10^{-3}, E_s and E_p are the enrichments of the final and predose samples. The final step in the equation, division by 1.01, is necessary since the ^{18}O dilution space is larger than TBW (6).

The mean daily CO_2 production (rCO_2, mole/day) is calculated according to Schoeller et al. (5):

$$rCO_2 = (N/2.078)\ (1.01k_O - 1.04k_H) - 0.0246rH_2O_f$$

where N is the average of the beginning and end of period total body water and rH_2O_f is the rate of water loss via fractionating gaseous routes, and is estimated to be $1.05N(1.01k_O - 1.04k_H)$. The ^2H and ^{18}O isotope elimination rates (k_H and k_O) are calculated using the initial and final time points (two-point method). In the Alaska90 Cold Weather Study and Bolivia90 high altitude study, linear regression using the isotopic enrichment relative to predose of the first two days and last three days of the metabolic study were also used to determine elimination rates. There has been some controversy regarding the accuracy and precision of the two-point and regression methods. The advantage of the two-point method is that we obtain the true elimination rate even during changing physiologic conditions (which often occurs in Army Field Studies). The advantage of the multipoint regression methods is improved precision from averaging out analytical error.

Energy expenditure is calculated by multiplying rCO_2 by the energy equivalent of CO_2 calculated from the macronutrient content of each diet, and body stores of protein and fat used for energy (7).

Isotopic analyses. The ^{18}O isotope abundances were measured on a Finnigan Delta S or Finnigan MAT 252 gas-inlet Isotope Ratio Mass Spectrometer with a CO_2-Water equilibration device. Briefly, urine and saliva samples were equilibrated with CO_2 at 18 °C in a shaking water bath for at least 10 h. The CO_2 is then cryogenically purified under vacuum before introduction into the mass spectrometer. The hydrogen isotope abundances were measured on a Finnigan Delta S or Finnigan MAT 252 gas-inlet Isotope Ratio Mass Spectrometer, as previously described (2). Briefly, urine and saliva samples were distilled under vacuum into tubes containing zinc reagent (Friends of Biogeochemistry, Bloomington, Indiana). The reduction tube were sealed with a flame and placed in a 500 °C oven for 30 minutes to reduce the water to hydrogen gas which is then introduced into the mass spectrometer.

Progress

Major Scientific Achievements

The major scientific achievements for the Stable Isotope Laboratory have been the measurement of energy in two Arctic Field Training Exercises, a high altitude training exercise and a Ranger Training Study using the doubly labeled water procedure. A summary of the projects is given in the table below. Detailed descriptions of the studies are presented in the following sections.

Study	Energy Expenditure (kcal/day)		
Alaska90	5170 ± 630		
Bolivia90	3550 ± 610		
Alaska91	4250 ± 480		
Ranger - 1991			
Fort Benning Phase	Shakeout	FTX	Camp Darby FTX
	5350 ± 770	3990 ± 820	3790 ± 1990
Mountain Phase	Climbing	"Classes"	FTX
	4550 ± 460	6050 ± 1540	4540 ± 800
Swamp Phase	3770 ± 1000		
Desert Phase	4330 ± 560		

Alaska90 Cold Weather Study

The deuterium and ^{18}O enrichment of 6 urine samples between February 4, and February 14 were analyzed in the six unlabeled subjects. There were no significant shifts in baseline isotope abundance in the unlabeled group. The deuterium and ^{18}O enrichment of 6 urine samples and 6 saliva samples were analyzed for the 14 labeled subjects. The elimination rates were calculated by the two point method, using the initial and final enrichments, as well as a regression method (5 time points). The analyses for one subject (#104) were repeated to determine the analytical precision. For ^{18}O the coefficient of variation for the elimination rate by the 2-pt method was 0.4% while for regression it was 0.8%. The CV for the dilution space was 0.4% and 0.1% for the initial and final time points. The elimination rates calculated by the 2 point method and the regression method were similar in some instances but considerably different in others. For deuterium, the coefficient of variation for the elimination rate by the 2-pt method was 1.2% while for regression it was 1.6% for the repeat analysis. As for ^{18}O the elimination rates calculated by the 2 point method and the regression method were similar in some instances but considerably different in others.

An average RQ and energy equivalent of CO_2 were estimated by calculating an FQ from the protein, carbohydrate and fat intake of each soldier (See 9th Quarterly Report). Energy expenditure was then calculated from the deuterium elimination rates and the ^{18}O elimination rates and dilution spaces which were determined last quarter. The average daily energy expenditure of the 14 labeled soldiers was 5143±595 kcal by the 2-point method and 4847±498 by the regression method. The reason for the difference between the two methods is that the regression method is sensitive to systematic changes in energy expenditure. In this study the soldiers were stood down on one day and underwent strenuous physical activity the next, and both of these time points were used in the regression analyses. When using the regression method, this causes the elimination rate obtained from the slope of the line to be skewed as well as the 0-time intercept used to calculate dilution spaces. These errors then cause an error in energy expenditure. The two-point method, however, is not affected and provides the true elimination rates. The dilution spaces are obtained by the plateau enrichment 4-hours after the dose.

Lean body mass (LBM), calculated by isotope dilution (TBW/.73) decreased from 64.9±5.3 at the beginning of the period to 64.3±5.2 by the end of the metabolic period. Body fat calculated from the difference between body weight and LBM only decreased by 1.8 kg during the study. This decrease is lower than the true fat loss, due to the timing of the final weighing. Initial weights were taken in the morning between 0600-0800 hours, while the final body weights were taken in the afternoon between 1500-1700 hours. Therefore, the final body weights would be higher than they would

have if taken first thing in the morning. Since fat weight is obtained by subtracting LBM from total weight, if the total weight appears higher than it truly is, then the fat weight will be overestimated. This fact is clearly demonstrated by the considerably lower energy expenditure calculated by the Intake/Balance (I/B) method. For this method the energy intake plus changes in energy content of the body (fat and protein) are combined to obtain energy expenditure. The average energy intake was 3059±784 kcal, the change in fat and protein energy content were 684±2781 and 82±327 kcal, giving an energy expenditure of only 4316±1087 kcal/d, which is over 800 kcal/d lower than that obtained by doubly labeled water.

Water intake determined by deuterium elimination, correcting for atmospheric water intake, isotope fractionation and estimated metabolic water production was similar to that obtained by dietary log information (3.4±0.03 vs 3.8±2.2 liters/day).

Bolivia High Altitude Study

The deuterium and ^{18}O enrichments of urine samples (6 and 5 time points respectively) of 6 subjects in the placebo group of the Bolivia high altitude study have were analyzed. Unlike the Alaska90 study, there was a significant decrease in enrichment over time. There was a shift in ^{18}O of -2.33 o/oo and in deuterium of -23.8 o/oo. The average decrease was used to correct the enrichment of the labeled subjects when calculating elimination rates. This correction is needed to obtain the correct energy expenditure during changing isotopic enrichment of the water supply. The deuterium elimination rate (Kd) is the average of two separate analyses for each subject. The deuterium analyses were repeated for two reasons: 1) because this was the first full project for the technician, and 2) to determine the accuracy of the determination of Kd, because deuterium is the most difficult analytical aspect of the method.

The calculations using the 2pt and regression method yielded almost identical means and standard deviations, 3549±608 vs 3565±674. The energy expenditures calculated from the repeat deuterium analyses also yielded nearly identical energy expenditures using either the 2pt or regression methods, with a coefficient of variation of about 2% (See 11th Quarterly Report). There were some problems with samples from subject #59, both in energy expenditure and total body water, and hence, was excluded from the mean calculations. The energy expenditure results have been published in USARIEM Technical Report No. T10-91 (8).

Alaska91 Cold Weather Study

The deuterium and ^{18}O enrichments of urine samples of 5 subjects in the placebo group were analyzed. As in the Alaska90 study, there was no significant change in enrichment over time.

Since the data from the Bolivia study demonstrated that the energy expenditure obtained using the 2pt and regression methods yield the same results, only the 2pt method was used in this study. The mean energy expenditure of the 10 labeled soldiers was 4250 ± 480 kcal/d. There was a problem with the first measurement of total body water for 4 of the subjects, it appears that there were problems with the measurement of the ^{18}O dose the soldiers received and in 2 cases problems with collection of saliva samples. Therefore, the RQ was assumed to be 0.823 and the estimate for the energy equivalent of CO_2 was 5.8 kcal/L.

Water intake determined by deuterium elimination, correcting for atmospheric water intake, isotope fractionation and estimated metabolic water production was similar to that obtained by dietary log information (3.1±0.08 vs 3.6±0.5 liters/day).

Ranger Training Study - 1991

There were four phases of the Ranger study: the Fort Benning phase (7/26/91 - 8/10/91), the Mountain phase (8/11/91 - 8/28/91), the Swamp phase in Florida (8/29/91 - 9/13/91), and a final Desert phase (9/14/91 - 9/26/91). The samples for this study will be analyzed as each phase of the study is completed.

There were logistical problems with the Ranger Study due to the high dropout rate. The original (optimistic) plan was to follow 6 soldiers through the entire training program, but only one of the soldiers chosen to receive label completed the program, and 3 soldiers completed three of the four phases. Data were obtained from 6 soldiers during Phases 1 and 3, and from 5 soldiers during Phases 2 and 4. Urine samples from a placebo group were analyzed for deuterium and ^{18}O, to correct for any changes in isotope abundance of the drinking water of the soldiers due to the changes in location. The summary for each of the isotopes and energy expenditure is given in the 14th Quarterly report. The mean change in isotope abundance for Phase 4 were used to correct isotope enrichments for the labeled subjects. The deuterium and ^{18}O raw data are included in the Appendix. In addition to energy expenditure, when carrying out the doubly labeled water method, water turnover (intake) can be calculated from the deuterium turnover data and total body water.

Energy expenditure during the Ranger Training Study was very high, with an mean of 4350 kcal/d for the days covered by the doubly labeled water periods. Mean energy expenditure was as high as 6045±1537 kcal/d during the "Classes" portion of the Mountain Phase. The isotope enrichments were too low to obtain reliable data for the end of the Fort Benning Phase, particularly the 8/6 to 8/10 portion. Even some of the 8/6 time points were very low, leading to less reliable measures of energy expenditure. This was a particular problem for some subjects, making the energy expenditure for the time points after 7/31 extremely suspect. It was interesting to

note that for one subject who dropped from the study and had barracks/grounds duty had an energy expenditure considerably lower than the mean energy expenditure for the periods. The isotope enrichments for the other Phases was high enough to obtain reliable data. The mean #energy expenditures were 4197±1052, 4978±775, 3769±998, and 4328±555 for Phases 1 to 4.

The preliminary water turnover results indicate very high water intakes, particularly during the Fort Benning Phase. This high water turnover led to the low isotope enrichments which caused problems in energy expenditure measurements late in the period. The mean water turnover was 8.5±1.3, 5.9±0.6, 6.6±1.3 and 5.1±1.7 liters per day for Phases 1 to 4. Surprisingly, the water turnover was lowest during Phase 4, the desert phase.

Water Turnover Studies

There have been two studies in which only water turnover has been studied. The first was part of the Fairchild Air Force Base Survival Study. There were 10 soldiers participating in the first iteration and 16 soldiers participating in the second iteration of the Survival Study. The initial and final deuterium enrichments for calculation of isotope dilution space (and total body water), and the deuterium elimination rate for calculation of water turnover were calculated. The results were reported in the 13th Quarterly report, and sent to Tanya Jones for final calculations.

The second study was the Wolf Creek Study, done at the Marine Mountain Warfare Training Center, Bridgeport, California. Water turnover was studied using ^{2}H labeled water. Urine and saliva samples from 1/24/92, and 1/31/92, and urine samples from 1/28/92, of 26 soldiers with complete samples were analyzed and sent to Tanya Jones for final calculations.

The following paragraphs describe planning activities that occurred during this grant period. The research proejcts, if executed, would be funded from another source.

Ongoing Projects

Discussions have been carried out with Col. Askew regarding planning for total body water and water turnover measurements for the upcoming Pikes Peak Study.

Discussions have been carried out regarding the collaborative study with the Israel Military Services. A letter has been received from Major Burstein of the IDF Medical Corps, Institute of Military Physiology. Samples from the "Mountain Phase" were collected in February 1992 and have been analyzed by Dr. Andy Coward at the Dunn Nutrition Laboratories, in Cambridge, U.K. The summer study will begin in August, and samples will be shipped to the Pennington Center for analyses of ^{18}O and ^{2}H.

Planning for the Ranger 2 Study has been carried out with Dr. Reed Hoyt. The dosing schedule for ^{18}O and ^{2}H, and the sampling time points were listed in tables in the 16th Quarterly Report. To obtain estimates of isotope elimination, water turnovers used were similar to those observed during the last Ranger study. The estimated energy expenditures were slightly higher than last years study, since in the present Ranger study, the soldiers will be given more calories and worked even harder than last year.

Conclusions

The doubly labeled water method has proven to be an ideal method for the measurement of energy expenditure of soldiers during field training exercises. The two-point method, in which elimination rates are measured from isotope enrichments of urine samples from the first and last days of the study has proven to be valid in these studies. Additional time points have been taken to break down a period into smaller intervals, such as in the Ranger Training Study. The only requirements of the soldiers is give urine and saliva samples and drink the heavy water.

The energy expenditure of soldiers during the Arctic Field Training exercises was higher than anticipated, particularly in the Alaska90 study. The energy expenditure during the Alaska90 study were considerably higher than during the Alaska91 study (5170±630 vs 4250±480 kcal/d). This is not surprising in light of the facts that it was considerably colder during the Alaska90 study, the soldiers were more active and needed to wear snowshoes more during the Alaska90 study, and the soldiers did not move their artillery as much as had been anticipated during the Alaska91 study. Therefore, energy requirements during Arctic exercises appear to be between 4000 to 5200 kcal/d. The MRDA of 4500 kcal/d during cold weather appears to be adequate, since in only one instance has expenditure been shown to be above this level (Alaska90), and soldiers have not eaten more than 3500 kcal/d, even when carrying over 6000 kcal/d into the field.

The energy expenditure at altitude during the Bolivia study was essentially the same as was anticipated. Energy expenditure and water turnover during the Ranger Training study were high, as expected.

References

1. Lifson, N. Theory of use of the turnover rates of body water for measuring energy material balance. J. Theoret. Biol. 12:46-074, 1966.

2. DeLany James P., Dale A. Schoeller, Reed W. Hoyt, E. Wayne Askew, and Marilyn A. Sharp. Field Use of $D_2{}^{18}O$ to Measure Energy Expenditure of Soldiers at Different Energy Intakes. J Appl Physiology 1989;67:1922-9.

3. Prentice, A.M., A.E. Black, W.A. Coward, G.R. Goldberg, P.R. Murgatroyd, J. Ashford, M. Sawyer, and R.G. Whitehead. High levels of energy expenditure in obese women. Br. Med. J. 292:983-987, 1986.

4. Schoeller, D.A. Use of two-point sampling for the doubly-labeled water method. Human Nutrition:Clinical Nutrition 38C: 477-480, 1984.

5. Schoeller DA. Measurement of energy expenditure in free-living humans by using doubly labeled water. J Nutr 1988;118:1278-89.

6. Schoeller, DA, E van Santen, DW Peterson, W Dietz, J Jaspen and PD Klein. Total body water measurement in humans with ^{18}O and ^{2}H labeled water. Am J Clin Nutr 1980;33:2686-93.

7. Lusk, G., The elements of the science of nutrition (4th ed.). New York: Academic, 1928, p6.

8. Edwards John S.A., E.W. Askew, N. King, C.S. Fulco, R.W. Hoyt, and J.P. DeLany. An assessment of the nutritional intake and energy expenditure of unacclimatized U.S. Army soldiers living and working at high altitude. USARIEM Technical Report T10-91, Natick, MA: U.S. Army Research Institute of Environmental Medicine, June, 1991.

9. King, N., S. Mutter, D.E. Roberts, E.W. Askew, M.Z. Mays, A. Young, T.E. Jones, B.E. Cheema, M.R. Sutherland, and J.P. DeLany. Nutrition and hydration status of soldiers consuming the 18 man arctic tray pack ration module with either meal, ready-to-eat or the long life ration packet during a cold weather field training exercise. USARIEM Technical Report T4-92, March, 1992.

III. Nutrition and Military Performance Laboratory

The work of the Nutrition and Mental Perofrmance has been primarily supported by the Department of the Army Grant DAMD 17-88-Z-8023.

Major Scientific Achievements, 1989-1992.

* First development of an antibody to dopamine D_2 receptor protein.

* First evidence that the dopamine D_2 receptor is coupled to a subunit of GTP-binding protein.

* First evidence that transcriptional regulation has only a minor, or secondary, role in the expression of dopamine D_2 receptors in the brain.

* First quantitative evidence that long-term, high dietary-protein results in an increase in motor activity and increased basal arousal levels.

* First definitive evidence that dietary protein influences the cellular morphology and functional state of the cerebral cortex.

* First to devise the methodology for separating and measuring all known serotinin metabolites in a single analysis.

* First definitive evidence that circulating cyclo(His-Pro), a neuropeptide that is known to modulate many types of behavior, is not derived from dietary protein.

* First evidence of a tonal discrimination task performed by anesthetized animals.

Background and Introduction

The focus of the Pennignton Center's neuroscience program is to apply the expertise of the researh staff to investigate the role of nutrition in behavior. Projects have been undertaken which include behavioral, neurophysiological, and molecular neurobiological measurements to study the effects of macronutrient manipulations on higher brain function. Overall, the research has broad application to problems related to aging and development, mental function and dysfunction, as well as to the questions of nutrition science. In the interest of taking a state-of-the-art, multidisciplinary approach to solving scientific problems, Dr. Prasad has assembled a team of scientists drawn from different specialities and possessing expertise in a variety of scientific methods. The scientific staff has included: Shakeel M. Farooqui, Ph.D. in Biochemistry, with expertise in Molecular Biology; Jeffery W. Brock, Ph.D. in Physiology, with expertise in Neurophysiology; Emmanuel S. Onaivi, Ph.D. in Pharmacology, with expertise in Behavioral Pharmacology; Anwar Hamdi, M.D., and Ph.D. in Pharmacology, with expertise in Behavioral Pharmacology; and Masahiro Sakata, M.D., with expertise in Molecular Biology. Students who have worked with the Neuroscience Lab at different times are: C. Z. Chuang, Lisa Theriot, Joseph LaFleur, Ashley Cowart, Stephanie Tarlton, Sheila Venugopal, Shorye Payne, Keith Ross, and Lori Adams.

The Nutrition and Mental Performance Laboratory research program has focused on basic and applied research, utilizing a number of techniques in molecular biology, neurochemistry, pharmacology, and neuro- physiology. The summary of the basic and applied research, and their application are presented below.

1) Applied Research

* Diet, brain chemistry, and behavior
* Nutritional factors in drug abuse
* Higher brain function

2) Basic Research

* Regulation of dopaminergic neurons
* Neurochemistry
* Molecular biology
* Neuronal function

The studies which have been performed over the past two years are topically related to problems of:

* Mental performance, function, and dysfunction,
* Aging and development,
* Neurological and mental disorders eg., Parkinsonism and schizophrenia

The Nutrition and Mental Performance Laboratory has enhanced its methodological capabilities by joining in collaborative research with the Gene Expression Laboratory and the Analytical Laboratory at the Pennington Biomedical Research Center. The rewards have not only been an advancement of our own research efforts, but a demonstration of our value as a resource for others working in the area of nutritional neuroscience.

General Progress.

Project 1: Behavioral Neurochemistry of Food-derived Peptides (project coordinator: Chandan Prasad). We have chosen three peptides to be included under this program: i) cyclo(His-Pro), CHP, ii) caesin-derived peptides (exorphins), and iii) delta-sleep inducing peptide, DSIP (a peptide known to reduce blood pressure and protect against stress response). The first phase of this study has largely concentrated on i) The relationship between diet and endogenous cyclo (His-Pro) levels, and ii) the mechanism of action of cyclo (His-Pro) in the striatum, an area of the brain actively involved in motor coordination.

CHP has been shown to exist in a variety of tissues and biological fluids such as the brain, GI tract, blood, CSF, and semen, etc. While CHP, like immunoreactivity from such biological specimens has been characterized chromatographically, in no case has the peptide been isolated in enough quantity and purity that its presence can be ascertained by physical methods. We for the first time, have isolated pure CHP from human urine and determined its structure to be histidyl-proline diketopiperazine. These data have been accepted for publication in "Biochemistry International".

Having established the existence of CHP in a biological fluid, we have focused our attention on the question "could dietary proteins serve as cyclo (His-Pro) precursors?". To this end, we have examined the urinary levels of CHP in three species- a carnivore (leopard), an herbivore (rhinoceros), and an omnivore (man). The data from these studies suggest that urinary levels of CHP is higher in animals consuming high levels of dietary proteins. However, in these studies data on exact composition of diet at dietary levels CHP was not available. Therefore, we subjected rats to three different diets (of known chemical composition with undetectable level of CHP): carbohydrate-rich, caesin-rich, and whey-rich.

Proteins in casein and whey have 13 and 3 Pro-His or His-Pro sequences. If both of these proteins were to be hydrolyzed in such a way to release all His-Pro of Pro-His sequences, animals on caesin-diet should excrete at least 4 times more CHP than those on whey diet. This hypothesis is also consistent with the observation that exogenous CHP rapidly clears from the plasma and accumulates in the urine. However, the results from this study show that the differences in the plasma or urine levels of CHP in rats on these three different diets (carbohydrate, caesin, and whey) were insignificant. In conclusion, it appears that endogenous CHP may not be derived from the metabolism of ingested dietary proteins.

In a related study, we have examined the presence of CHP in 12 common nutritional supplements using partial protein hydrolysates. Nine out of 12 samples contained CHP. Those supplements with the highest CHP levels had undergone more intense thermal manipulation prior to packaging than others. Furthermore, oral administraiton of one of these supplements (Ensure) to a human volunteer resulted into a rapid rise in plasma levels of CHP. In conclusion, these data show that while it is unlikely that CHP may be derived from dietary proteins, a diet containing hydrolysed protein (or CHP) may contribute to endogenous levels of CHP.

Project 2: Cyclo(His-Pro) and food intake (project coordinators: Anwar Hamdi and Jeffery Brock). Administration of exogenous CHP to rats and mice has been shown to elicit many endocrine and central nervous system-related biological activities. CHP in a dosage of 2.5 uM/rat/day is known to produce a 20% ($p<0.05$) reduction in daily food intake. Consistent with the appetite-modulating effects of the peptide is the observation that fasting elevates the hypothalamic CHP content which returns to normal after feeding. Until recently, the inhibitory effects of CHP on food intake had been demsontrated using a mixed diet only. It is well known, however, that rodents can regulate their macronutrient intake when presented separately with carbohydrate (C), protein (P), and fat (F) diets. This led us to investigate whether intraventricularly administered CHP may affect caloric intake and, if so, would the changes in caloric intake be due to

alterations in the intake of all or only some of the macronutrients.

To accomplish this, rats were allowed to choose from C-, P-, and F-rich diets to display macronutrient preferences after vehicle or CHP infusion into the cerebral ventricles. Nintey percent of the calories in C-, P-, and F-rich diets were derived from C, P, and F, respectively, with the remaining ten percent of the calories derived from equal parts of the other two macronutrients. Fisher 344 rats (400-450 gm) were implanted with indwelling intraventricular canulae, housed individually, and then allowed to recover from surgery for 4-5 days. Rats were fasted from food, but not water, for 21 hours and then allowed to consume three macronutrients presented separately for a total period of 3 hours. Total energy intake (Kcal/Kg/3 hrs) and percent of total energy derived from each macronutrient was calculated on five consecutive days prior to vehicle or peptide (CHP, 1-methyl CHP, or 3-methyl CHP) administration.

Administration of saline (vehicle) alone led to an appreciable increase in total caloric intake, which was characterized by increased preference for fat and decreased preference for both carbohydrate and protein. These changes after vehicle administration may be due to non-specific stress resulting from handling and intraventricular perfusion. On intraventricular administration of CHP (0.5 uM/Kg), but not 1-methyl CHP or 3-methyl CHP, both total caloric intake ($p=0.0075$) and fat preference ($p=0.0354$) decreased, whereas carbohydrate preference increased ($p=0.0518$), with no change in protein preference ($p=0.2458$). In conclusion, these data show that CHP differentially modulates macronutrient selection. Therefore, endogenous CHP in the central nervous system may play a role in regulation of food preferences.

Project 3: Determination of tryptophan metabolites using HPLC (project coordinators: Chandan Prasad and C. Z. Chuang). The amino acid tryptophan is an initial substrate for brain serotonin synthesis which is not easily transported across the blood brain barrier. Serotonin activity potentially has applied consequence throughout the CNS. Thus, the study of the pathways for tryptophan metabolism is critical to an understanding of the cerebral serotonin function in a number of behavioral systems. There are at least two major pathways for tryptophan metabolism. The first pathway leads to the decarboxylation of tryptophan. The second pathway, the pyrotase pathway, results in the formation of metabolites following cleavage of the indole ring. At least two of the metabolites of the tryptophan pyrotase pathway - quinolinic acid and kynurenic acid - have been reported to play important roles in excitatory neurotranmission, neurotoxicity, and epilepsy. Kynurenine has also been shown to act as an excitotoxin in in vitro studies.

Studies into the role of other tryptophan metabolites in the pathogenesis of human neurological disorders are limited by the technology available to separate and quantitate these compounds in biological samples. Quinolinic acid, a tryptophan metabolite with a major role in CNS function, has generally not been included with other metabolities in previous analytical procedures; therefore there is a need for a new sensitive method that can separate and measure many metabolities, including quinolinic acid, in a single sample. In order to optimize the conditions for the simultaneous separation of the tryptophan metabolites, a reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed. This involved the sequential optimization of the mobile phase, by adjusting the pH, the concentration of of triethylamine and the gradient elution. The baseline resolution of the compounds by this optimized procedure was obtained with an anlysis of time, including the re-equilibration period of less than 30 minutes. This research, which has been accepted for publication in the Journal of Chromatography, is the first RP-HPLC method that can separate tryptophan and its metabolites in a single chromatographic run. A copy is enclosed as the appendix.

Project 4: Preparation and characterization of dopamine D_2 receptor protein antibody (project coordinators: Shakeel Farooqui and Jeffery Brock). Dopamine plays an important key role in brain function. The abnoramalities in the metabolism of dopamine in specific regions of the brain leads to mental and neurological disorders , which are characterized in schizophrenia and Parkinson's condition. In order to study these molecular disturbances we raised antibodies against the dopamine receptor type D_2 in rabbits. Two peptides corresponding to amino acid sequence predicted from the nucleotide sequence of the dopamine D_2 receptor were chemicaly synthesized. Peptide 1 (CGSEGKADRPHYC) and Peptide 2 (NNTDQNECIIY) corresponding to 24-36 and 86-98 from the NH2 terminal. The peptides were conjugated with a keyhole limpet hemocyanin using glutraldehyde and the conjugate was injected to rabbits. The polyclonal antiserum was obtained and screened for specific antipeptide 1 or antipeptide 2 antibodies on ELISA. Antibodies against peptide 1 showed high titer for peptide 1 with little or no cross reactivity with the other peptides. The antibodies were further characterized on a Western blot. Peptide 1 antibodies reacted with denatured D_2 receptors from rat striatal membranes, Mr 91 kDa. The preimmune sera or peptide 2 antibodies did not show any band corresponding to 91 kDa. Peptide 1 antibodies were further characterized for immunoinhibition studies using D_2 specific ligands. Peptide 1 antibodies significantly (40%) inhibits the photoaffinity labeling of D_2 receptor by 125I-NAPS. Such an interaction of antibody with native D_2 receptor was further studied using a D_2 specific ligand (3H) YM-09151-2. (3H) YM-09151-2 binding was significantly inhibited (35-40%) by the addition of peptide 1 antibodies. The addition of preimmune or pooled rabbit serum did not show an inhibition in the YM binding. These results suggest the presence of anti D_2 receptor antibodies

which binds to dopamine receptor either on the ligand binding site or in close proximity, which results in the inhibition of ligand-receptor interaction.

Project 5: Determination of dopamine (D2) receptor messenger RNA expression (project coordinators: Masahiro Sakata and Shakeel Farooqui). The application of this technology has a major contribution in the study of central dopaminergic mechanisms which are implicated in a number of neurological and mental disorders. Our laboratory has made rapid progress in establishing the protocol for the determination ofthe dopamine, D2 receptor mRNA expression using Northern blot analysis. Using the modified guanidium thiocyanate method, the total mRNA extracted was denatured at 55 C in 50% formamide, (6% formaldehyde solution for 15 minutes and electrophoresed in 1% agarose / 0.66M formaldehyde gels. After electrophoresis, RNA on the gels was transferred to nylon filters and the filter was baked at 80 C for 2 hours. The filter was prehybridized for one hour at 42 C in 50% formamide, 0.25M NaCl, 0.25M sodium phosphate (pH 7.2), 1mM EDTA and 0.1% SDS in a volume of 10ml and hybridized for 24 hours at 42 C with 1.0x106 c.p.m./ml 32-P-labeled PD2 cDNA in the same hybridization buffer. The membranes were washed at room temperature in a solution containing 2xSsc and 0.1% SDS (200ml) and then twice at 65 C in 200ml 0.1ml 0.1xSSC and 0.1% SDS. Autoradiography was done at -70 C for one day. All northern blots were probed also for B-actin mRNA content, as control.

The D2 mRNA that was detected using the PD2-DNA probe was 2.6kb and was prominent in pituitary and striatal tissues, but was not seen in the testes and liver. As positive control using the B-actin probe, strong bands were detected following hybridization in all samples indicating a successful RNA preparation. With this capability, a number of problems can now be investigated in the laboratory, such as: 1) the expression of D2 mRNA following dietary and other physiological and pharmacological manipulations relevant to the aging process, 2) the mechanism of regulation of the D2 mRNA expression in animal models and its control, and 3) dopamine agonists and antagonistic effects and the effects of dietary manipulation on the D2 mRNA are to be determined.

Project 6: Dopamine D2 receptor protein antibody mapping in the rat brain (project coordinator: Jeffery Brock). This study was undertaken to demonstrate the utility of the antibody as a D2-selective label by determining the distribution of antibody binding in situ. Rat brains were dissected and sliced on a freezing microtome into 40 micron sections. Every fourth section was incubated with D2-selective antisera, following the procedure of peroxidase-anti-peroxidase (PAP) immunocytochemical labelling. Alternate sections were incubated with pre-immune sera and antisera plus free oligopeptide. The anatomical distribution of PAP complex was verified in selected sections which were stained with cresyl

violet, and compared to Paxinos' "The Rat Brain in Stereotaxic Coordinates".

PAP complex staining was heavy in the striatum, frontal and parietal cortices. PAP complex was distributed throughout the basal forebrain and stained the heaviest in the olfactory tubercle, medial septal nuclei and nuclei of the diagonal band In the hypothalamus, lighter, diffuse staining continued throughout, with heaviest precipitate in the periventricular area. Staining was evident throughout the medial forebrain bundle all the way back to the ventral tegmental area and the substantia nigra pars compacta. Heavy staining was observed in the dorsal thalamic nuclei and in the medial habenula, and more caudally in the midbrain, the central gray area around the Aquaduct of Sylvius. Unexpectedly, PAP was seen in lateral thalamic nuclei, also in the superior and inferior colliculi. This previously undescribed distribution of D2 receptors may belong to the periaquaductal dopaminergic system, which have their cell bodies of origin in the zona compacta and send afferents to the dorsal midbrain.

Sections incubated with primary antiserum followed by free peptide antigen in concentrations greater than 10 microgm/ml showed no precipitate in any of the above mentioned areas. Inhibition of specific antibody binding in this way resulted in little or no contrast staining, and an appearance identical to those sections incubated with pre-immune serum. These data are consistent with the distribution of efferent dopaminergic fibers, and the binding distributions of known D2 receptor radioligands. Also, these data are in good agreement with the areas known to express D2 mRNA, which has been found in the highest concentrations in the neostriatum, olfactory tubercle, substantia nigra, ventral tegmental area, nucleus accumbens, and the intermediate lobe of the pituitary gland. These data suggest that D2 receptor protein was recognizable at all levels of the dopaminergic system, i.e., target tissue, axons, and cell bodies of origin.

Project 7: Dietary protein and behavior in rats (project coordinators: Emmanuel Onaivi and Jeffery Brock). Many studies indicate that dietary macronutrients are important determinants of brain function. Both undernutrition and overnutrition in preweaning rats result in persistent functional alterations of the brain. The effects of protein undernutrition on behavior, brain development, and intellectual function are well known. Unfortunately, reports on the effects of high dietary protein on animal behavior are largely anecdotal. For example, it has been observed that adult rats consuming a high-protein diet become more easily frightened and "snappish". In addition, much of our knowledge about the effects of high dietary protein on behavior is inferred from studying the effects of tyrosine administration, based upon the understanding that brain tyrosine is increased by a single meal in proportion to its protein content. The issue is complicated by the observation that brain levels of tyrosine are

not increased with long-term consumption of high dietary protein, apparently due to an adaptational decrease in total intake by the animal. Thus, tyrosine administration may not accurately model the effects of long-term, high-dietary protein on behavior.

In order to better understand the role of dietary protein in behavior, more precise and quantitative assessments of behavior must be made in animals consuming different levels of protein. Investigators currently have a battery of behavioral tests at their disposal which are both quantitative in nature and, in some cases, characterized with regard to known neurotransmitter(s) involved in mediating the behavior. The adhesive patch test and negative geotaxis are commonly used to evaluate sensorimotor function in rats; locomotor activity and stereotypy are inversely related measurements of ambulatory, or searching, behavior in rats; the tail-flick test is widely used to measure nociception in rats and mice; activity in the elevated plus-maze is a well characterized index of aversion behavior and anxiety. In the present study, these tests were used to evaluate the effects of varying levels of dietary protein on behavior in adult rats.

In the first phase of these experiments, groups of rats were pair-fed with isocaloric diets containing normal-(20%), low-(8%) and high-(50%) casein for 20 weeks. In assessing the performance of rats in the battery of behavioral test systems the animals on the high-protein diet were more responsive in sensorimotor function, negative geotaxis and spontaneous locomotor activities when compared to normal and the low protein groups. These rats showed a reduced aversion in the elevated plus-maze test which has been extensively used to study anxiolytics and anxiogenic drugs. In the tail-flick reaction time to a heat stimulus, analgesia was produced in animals fed the low-protein diet while hyperalgesia was induced in animals on the high-protein diet.

Project 8: Levels of dietary protein and modification of behavioral responses to CNS acting drugs (project coordinator: Emmanuel Onaivi). An elaborate study was undertaken to determine the effects of long-term dietary protein manipulation on the behavioral effects of some centrally acting drugs. In this study, mice were used and placed on one of the three isocaloric diets for 35 weeks: high-protein (HP), normal-protein (NP), low-protein (LP). The diets consisted of 50, 20, and 8% casein, respectively. The rest of the calories in the diet were made up with cornstarch and sucrose, and equal amounts of fat. All three diets were supplemented with salt and vitamin mixture and choline bitartrate. During the treatment period, the animals' body weights were not significantly different in the three groups. Locomotor activity and stereotypy following the administration of the vehichle or amphetamine (0.1 and 1.0 mg/ml), which were measured using the opto-varimex mini system (Colunbus Instruments, Ohio). The data was analyzed using one-way ANOVA followed by Dunnett's q' test.

Both spontaneous locomotion and stereotypy increased as the level of protein in the diet increased ($p<0.05$, N=6 per group). The NP fed animals exhibited a slight decrease in locomotion at low amphetamine but significantly increased at the higher dose of amphetamine. In contrast, LP animals showed significant increase in locomotion at both amphetamine doses. The stereotypic response after amphetamine in the LP or NP animals exhibited similar pattern as the locomotor activity. These results suggest that central dopamine receptors were altered by the long-term dietary protein manipulation and consequently modified the amphetamine induced behavior.

The next series of experiments were designed to further assess the influence of the long-term dietary protein manipulation and the consequences on the behavioral performance following the administration of a neuroleptic, fluphenazine, and the psychoactive constituent of marijuana, delta-9-THC. In this experiment, male ICR mice weighing 20-25 grams were housed in a temperature controlled room with reversed 12:12 hr light/dark cycle. The animals in the following groups were fed equicaloric diets, A: low-protein (8% casein); B: high-protein (50% casein); and C: medium protein (20% casein) for 35 weeks.

Animals in the different groups were injected intraperitoneally (ip) with the vehicle, delta-9-THC, or fluphenazine. The performance of the animals in a number of behavioral test systems was evaluated following the administration of the vehicle of drug regimen: fluphenazine (0.01-0.5 mg/kg), delta-9-THC (1-30 mg/kg). The vehicle or drug were administered for 40 minutes prior to behavioral analysis. The spontaneous locomotor activity of mice was monitored in individual activity cages following vehicle or drug treatment. The computer-controlled system was designed to monitor the total as well as ambulatory counts. The stereotype response was deduced from the difference between the total and ambulatory counts.

The dietary protein manipulation modulated mouse motor behavior with the spontaneous locomotor activity of the animals on the high protein diet increased by about 50% ($p<0.05$). In naive animals, delta-9-THC or fluphenazine produced a dose dependent inhibition of mouse spontaneous locomotot activity. The high-protein diet increased the mouse sensitivity to the locomotor inhibitory effects of fluphenazine or delta-9-THC.

The Pertwee ring test was utilized to assess catalepsy and data expressed as an immobility index. All animal were assessed for a total of five minutes and the time each animal remained motionless on the ring was recorded. In naive mice, fluphenazine or delta-nine THC induced a dose dependent state of immobility. It was observed that the dietary protein manipulation modified the catalepsy induced by fluphenazine or delta-9-THC. The high protein

diet influenced the cataleptogenic sensitivity to fluphenazine or delta-9-THC, as compared to the low or medium protein fed animals.

The tailflick reaction time to a heat stimulus was determined after vehicle or delta-9-THC administration. A ten second maximum latency was set to prevent tissue damage. The change in latency for each animal was computed and expressed as % MPE (% possible effect), where % MPE was determined using the following method: {(test latency-control latency)/ (10 seconds-control latency)} x 100. The long-term dietary manipulation increased the mouse sensitivity to the effects of delta-9-THC.

The computer controlled two compartment black and white box, as well as the elevated plus maze was used to determine the stress/anxiety index following different diets. The exploratory activity in the black and white chambers as well as the number of transitions were recorded in a 5 minute test session. The feeding of the high- and low-protein diets reduced and increased, respectively, mouse aversion in this test which is known to be sensitive to anti-anxiety drugs. The dopamine antagonist, fluphenazine, induced catalepsy, inhibited stereotypy, and reduced mouse spontaneous locomotor activities. A similar pattern was recorded with delta-9-THC, demonstrating modification of murine behavior following manipulation of dietary protein.

The results taken together suggest that the CNS function which can be influenced by long term dietary protein alteration, may modify those receptors that are sensitive to the effects of delta-9-THC. Furthermore, the central dopamine receptor function was altered by the dietary protein manipulation. It is unlikely that the mechanism of action underlying the behavioral modification induced by delta-9-THC or fluphenazine following the dietary manipulation are the same.

Project 9: Dietary protein and dopamine receptor regulation (project coordinators: Anwar Hamdi and Emmanuel Onaivi). We have collected a large body of evidence that dietary-protein manipulations have definite effects on higher brain function in animals, with evidence accumulating from behavioral, neurophysiological, and neurochemical studies. Preliminary data from our lab suggests that these effects include changes in neurotransmitter receptor populations in the central nervous system. Binding data was collected using the rat striata of the 8% casein, 20% casein, and 50% casein diet groups. The data indicated a 30% decrease in D2 receptor binding in the group of animals that were fed the low protein diet. The low-protein diet group also demonstrated a reduction in striatal dopamine receptor protein compared to controls, as indicated by D2-selective antibodies recently developed by our lab. Animals which were fed the high-protein diet demonstrated more dense antibody binding on immunoblot, suggesting that more dopamine receptor protein was present in the sample. Strangely, in the binding studies, Bmax for

the high-protein group was not different from control. In total, there is evidence that central dopaminergic mechanisms may be respectively facilitated or reduced by an increase or decrease in dietary-protein levels.

Project 10: Dietary protein and preparatory arousal in rats (project coordinator: Jeffery Brock). Previous investigators have observed that rats fed high-protein diets (50-80% casein) are easily frightened and demonstrate more violent behavior than rats on control diets. Data from our laboratory has shown that rats fed a chronic, high-protein diet (50% casein) are more reactive to nociceptive stimuli than those fed either normal-or low-protein diets (20 and 8% casein, respectively). The mechanisms underlying these changes are unknown. One theory is that high-dietary protein increases tyrosine availability for the synthesis of central catecholamines which, in turn, increase arousal levels in the animal. A weakness of this theory is that tyrosine levels are not elevated in the rat brain at dietary-protein levels of up to 80% casein.

The Cortical Negativity Response is an electrical correlate of the Alerting Reaction and preparatory arousal levels. This negative shift in cortical slow potentials is easily recordable even in anesthetized animals, when the animal is conditioned using an alerting stimulus-imperative stimulus paradigm. Cortical Negativity Responses were successfully recorded in urethane/chloralose anesthetized rats which were fed either a 20% or 50% casein diet for 36-40 weeks. There were two identifiable negative deflections, designated N1 and N2, which occurred after the alerting stimulus and before the imperative stimulus. Each peak was analyzed with regard to latency, amplitude, and duration. N1, which is generated by the frontal cortex as an orienting response to alerting stimuli, was not different between the two diet groups. However, the N2 deflection, which is generated by the motor cortex, was significantly prolonged in latency and higher in amplitude in the high-protein diet group. It is known from primate studies that the amplitude of this deflection is related to the subject's basal arousal level and the subject's preparation, or intention, to move. Furthermore, N2 amplitude is directly correlated with dopaminergic activation in the central nervous system. Additional testing revealed no differences between groups with regard to somatosensory evoked potentials and short-latency brainstem auditory evoked responses. These results suggest that the high-protein diet caused an increase in preparatory arousal mechanisms, which was not accompanied by changes in sensory information processing. These data are consistent with the theory that high-protein diets cause an over expression of catechol-aminergic mediated behavior.

Project 11: Dietary protein and neuronal plasticity (project coordinator: Jeffery Brock). In the relatively short time that the Neuroscience Laboratory has been operational, we have collected a large body of evidence that dietary-protein manipulations have effects on higher brain function in animals. There is reason to believe that central dopaminergic mechanisms are facilitated or inhibited, respectively, by an increase or decrease in dietary-protein levels. The available evidence has accumulated from behavioral, neurophysiological, pharmacological, and neurochemical studies. Such a multi- disciplinary approach is necessary, given the complexity of the brain; no one method can provide sufficient information to describe adequately the changes that occur after nutritional manipulations.
Combining descriptive morphology with neurotransmitter level measurements, etc., may provide further insight into the complex processes underlying behavior.

Changes in neurotransmitter and postsynaptic membrane protein synthesis alter the functional properties of synapses. These may be accompanied by alterations in synaptic structural properties as well. Although functional deficits of the brain can be caused by changes which are not reflected in gross morphology, recent works have shown that synaptic densities in certain areas of the brain are modified by nutrition.

Under certain conditions, synapses show plasticity, i.e., a change in their pattern of structural or functional connectivity. In the cerebral cortex, 80-90% of axons terminate on dendritic spines. In general, dendritic spines constitute as much as 96% of the surface area of pyramidal cells in the cerebral cortex and it is now well-accepted that dendritic spines play an important role in mechanisms of behavior, including learning, general states of alertness, and mentation. The purpose of the present study is to determine if the dramatic functional and biochemical changes which result from manipulating dietary protein are accompanied by changes in dendritic spine density. This is important because a decrease in dendritic spine density, i.e., a deficit in synaptic connections, would provide a simple causal explanation for the relationship between nutrition and functional impairment.

The Rapid Golgi method, which involves incubating brain tissue with osmium dichromate and silver nitrate, offers the opportunity to view single neurons with all their processes stained. This stain penetrates to very few cells in the tissue, but makes visible the details of axonal and dendritic ramifications not seen with usual histological techniques. This allows one to compare fine details of dendritic morphology between normal and experimental animals.

The present study was undertaken to determine if the dramatic functional and biochemical changes which result from manipulating dietary protein are accompanied by changes in dendritic spine

density. Brains were dissected from different groups of rats which consumed 8%, 20%, or 50% casein diets for 4 weeks. The tissues were fixed in 10% formalin and sliced into 150 micron sections, using a freezing microtome. The sections were stained using the Rapid Golgi method and dendrites were visualized by Nomarski differential phase-contrast microscopy. Dendritic spine densities were determined for the following areas: frontal cortex, parietal cortex, entorhinal cortex, striatum, and septum. Spine densities were statistically analyzed using single factor Analysis of Variance, followed by Student's t-tests. Statistical significance was accepted at the 95% confidence level (alpha = 0.05, two-tailed test).

In animals maintained on 50% casein for 4 weeks, dendritic spine densities were significantly increased in all 5 brain areas investigated ($p<0.05$), compared to the control group (animals maintained on 20% casein). For the animals maintained on the 8% casein diet for 4 weeks, dendritic spine densities were only significantly different in the striatum and entorhinal cortex, being elevated in both areas compared to the control animals. In the animals consuming the 8% casein diet, spine densities in the frontal cortex, parietal cortex, septum were not different from control animals. These rather surprising results suggest that dendritic spine density is sensitive to levels of protein in the diet; however, the relationship between dietary protein and brain cell morphology is not a simple covariance. This non-linear effect on spine density induced by the dietary manipulation suggests that protein undernutrition and overnutrition stimulate different physiological mechanisms in the brain. The possibility is underscored by the observation that, not only did protein undernutrition and overnutrition have non-linear effects on specific brain areas, but the responses were also different between brain areas. These differences probably reflect the neural and biochemical individuality of each area.

Our understanding of the physiological roles of these specific brain areas in their contribution to behavioral expression provide for some interesting speculation as to the mechanisms invovled in the effects of dietary protein on behavior. In the case of the 8% casein-fed animals, increased spine density in the striatum and entorhinal cortex may reflect an increase in food-searching behavior in the animal while sparing general cognitive function. In the case of the 50% casein-fed animals, the over-development of dendritic spines in the cerebral cortex, striatum, and septum suggests a widespread increase in neuronal excitability in the brain. An increase in neuronal excitability in these key brain areas may be the neural substrate of the behavioral hyperactivity and hyper-responsiveness previously observed in animals maintained on long-term, high-protein diets.

Project 12: Dietary protein and microtubule-associated proteins (project coordinators: Shakeel Farooqui and Jeffery Brock). This investigation is intimately related to the subject of neuronal plasticity, mentioned above. Dendritic spines are dynamic structures that are capable of very rapid structural modification. These shape changes involve actin which is present in the spine in a microspecialized configuration that permits local contraction or extension of the cell membrane. Actin networks are isotropic gels and actin gel-solution transitions are under the control of local calcium concentrations. Others have shown that actin gel-solution transition may also involve the differential phosphorylation of microtubule-associated protein (MAP) subtypes, MAP1, MAP2, and MAP-tau. MAP2 is expressed exclusively in the brain and is highly localized in neuronal soma and dendrites, whereas MAP1 and tau are expressed throughout the cell. The phosphorylation of high molecular weight MAP2 appears to be a dendrite-specific event that is required for neuronal plasticity; it is beleived that interaction between MAP2 and actin allows for rapid cytoskeletal rearrangements within dendrites. In contrast, MAP1 and MAP-tau proteins are associated with stabilized (unchanging or non-plastic) cytoskeletal structures. Tau expression specifically is important for regulating the selective stabilization of microtubules accompanying extension of the neuronal cell membrane. Although phosphorylation of MAP2 itself is not sufficient to induce the formation of dendritic spines, the local expression of microtubule-associated proteins are useful indices of dynamic changes in dendritic surface area.

Rats were fed 8% (ad lib), 20% (pair-fed; equicaloric with 8% group), 20% (ad lib), or 50% (pair-fed; equicaloric with 8% group) casein for 36 weeks and their brains were collected. Selected areas of the brain (frontal cortex, entorhinal cortex, striatum, cerebellum) were dissected. MAP2 and MAP-tau proteins were solubilized and separated using 3-15% polyacrylamide gel electrophoresis. The blots were incubated with mouse monoclonal anti-MAP2 and mouse monoclonal anti-Tau antibodies. The results of the immunoblots are presented in the appendix (Figures 1 - 4). Both low (8%) and high (50%) casein diets resulted in increased expression of high molecular weight MAP2 (HMW-MAP2) in the frontal cortex and cerebellum, but diminished expression of HMW-MAP2 in the striatum and entorhinal cortex, compared to the equicaloric, 20% casein-fed group (Figures 1 and 2). Caloric restriction alone resulted in a dramatic increase in HMW-MAP2 in the striatum and entorhinal cortex, but a decrease in HMW-MAP2 in the cerebellum. In the striatum and cerebellum, these effects apparently were compensated when dietary protein was fed at both 8% and 50% levels. Caloric restriction had no effect on expression of HMW-MAP2 in the frontal cortex. Expression of MAP-tau proteins were not significantly altered by manipulation of dietary protein or caloric restriction (Figures 3 and 4).

Manipulating dietary protein and caloric restriction resulted in a complexed pattern of changes in MAP2 expression which must be interpreted cautiously. However, a few observations may be made from the data which are intrigueing:

1) Expression of HMW-MAP2 was sensitive to manipulations of dietary protein in all brain regions analyzed.

2) The frontal cortex was sensitive to manipulations in dietary protein, but apparently insensitive to caloric restriction.

3) Within the same brain region, high- and low-dietary protein resulted in qualitatively similar expressions of HMW-MAP2.

4) As a result of manipulating dietary protein, intracellular mechanisms associated with neuronal plasticity were enhanced in the frontal cortex and cerebellum, but diminished in the striatum and entorhinal cortex.

Project 13: Dietary protein and brain amino acid profiles (project coordinators: Jeffery Brock and Richard Tulley). High dietary protein in rats results in hyperactivity, hyper-responsiveness, anxiolysis, and increased basal arousal levels. These observations suggest a very complexed pattern of neurotransmitter and neuromodulator involvement. Firstly, hyperactivity and hyper-responsiveness suggest an over-expression of the central dopaminergic system in the brain. Interestingly, total dopamine content of the brain remains apparently unchanged by a 50% casein diet. However, analysis of discrete brain nuclei by punch dissection and HPLC/ED revealed that certain areas of the brain had significantly greater amounts of dopamine, while levels were diminished in other areas. Increases in dopamine should be accompanied by an increase in the availability of the amino acid, tyrosine, as precursor. Although others have shown that total brain tyrosine level is not significantly elevated by 50% casein diet, there remains the possibility that the differential increases in dopamine concentration may be accompanied by corresponding increases in tyrosine levels.

Hyper-responsiveness may involve under-expression of the serotonergic system. High dietary protein tends to reduce uptake of the amino acid tryptophan (serotonin precursor) into the brain. The observed hyper- and hypo-responsiveness in rats consuming high- and low-protein diets, respectively, are consistent with reports of decreased pain sensitivity in humans following tryptophan administration.

The phenomenon of anxiety, or aversion, behavior in rats is very complexed. Serotonergic system involvement is complex and controversial. Also, high dietary protein results in anti-aversive, or anxiolytic, behavior which is similar to the action of diazepam in humans. Diazepam is an agonist for part of the gamma-

aminobutyric acid (GABA) receptor complex. The implications are that GABA activity may be inhibited in certain areas of the brain, as a result of the high-protein diet, in a way which dis-inhibits the expression of behavioral reflexes. To make the story even more complicated, there is compelling evidence that inhibitory amino acids (GABA, glycine, taurine) and excitatory amino acids (glutamate, aspartate) may function to counter-balance each other, within highly localized anatomical domains, to control the expression of behavioral subroutines.

Rats consuming a high-protein diet also display an increase in basal arousal level. The mechanism for this observation is not known, although the observed amplification of cortical negativity responses is consistent with dopamine over-expression. The phenomenon also may involve an increase in energy expenditure, which may be revealed by alterations in brain levels of amino acids associated with the tricarboxylic acid cycle (glutamate, alanine) and reflect changes in cerebral protein metabolism (leucine, methionine).

The conclusion from this discussion is that it is imperative to determine the effects of dietary protein on rat brain amino acid profiles in order to elucidate the mechanism of protein-induced hyperactivity, hyper-responsiveness, and anxiolysis. With this objective in mind, rats were fed 8%, 20%, or 50% casein diets for 4 weeks and for 36 weeks, then sacrificed by rapid decapitataion, and their brains stored at -80° C until ready for processing. Selected areas of the rats' brains (frontal cortex, parietal cortex, occipital cortex, enhorhinal cortex, cerebellar cortex, striatum, septum, hippocampus, anterior thalamus, and hypothalamus) were dissected and processed for analysis. Samples of each area were taken into 600 microliters of 3% perchloric acid, weighed, and sonicated. Protein assays are presently being performed on the homogenates. The samples will be filtered (.45 um) and amino acids will be analyzed, using precolumn derivatization and HPLC, by the PBRC Analytical Laboratory.

Project 14: Duration of auditory memory traces in the rat brain (project coordinator: Jeffery Brock). An event-related potential called "stimulus mismatch negativity" is generated by the brain's automatic response to changes in repetitive auditory input. This response has been previously recorded only in awake humans and in sleeping cats. Investigators have postulated that varying the interstimulus interval during the stimulus mismatch negativity paradigm provides a valid measurement of the duration of auditory, short-term memory traces. Our laboratory has successfully completed the first recording of stimulus mismatch responses (MMRs) from urethane/alpha-chloralose anesthetized rats. Data analyses of MMRs in normal adult and aged rats have been completed.

Two groups of male, Sprague-Dawley rats were used in these studies. Groups 1 consisted of rats that were 7 - 11 months old (N

= 20) and group 2 consisted of rats that were 18 months old (N = 8). Each animal was anesthetized using alpha-chloralose and urethane (50 mg/kg and 1.5 gm/kg i.p., respectively). The body temperature was maintained at 37±.5 °C by a heating pad placed under the animal and monitored by rectal probe. A tracheostomy was performed and the animal was permitted to breathe spontaneously. The animal's head was fixed in a stereotaxic frame. Ear bar adaptors were positioned in the indention of the animal's squamosal bones. Earplug speakers were inserted into the left and right auditory meatus and secured in place with surgical tape.

Prior to the recording of MMRs, the functional integrity of the animal's auditory circuit was analyzed by recording brainstem auditory evoked potentials. Platinum wire electrodes were inserted subcutaneously over the skull. A reference electrode was postioned over the top of the skull at the midline. Active electrodes were positioned just behind each ear and lateral to the temporalis muscles. A ground electrode was positioned over the frontal bone. The auditory stimulus consisted of rarifaction clicks, 100 usec in duration, and at a rate of 11.4/sec. The stimulus was delivered binaurally at different intensity levels (35 and 75 dB nHL). Wave IV latencies were recorded and graphed to verify the integrity of the animal's auditory information processing.

For the recording of MMRs, platinum needle electrodes were placed in contact with the dura mater through small holes made in the skull. A reference electrode was located at Lambda. An active electrode was located 4 mm lateral over the right parietal cortex. A ground electrode was placed over the frontal cortex. A standard tone was delivered binaurally to the animal with a frequency of 4 kHz, an intensity of 90 dB nHL, a 1 msec ramp, and a 10 msec plateau. The standard tone was delivered with a 95% probability. The deviant tone was a 6 kHz frequency that was delivered with a 5% probability, but was otherwise identical to the standard tone. Electroencephalographic activity in response to the standard tones (950 sweeps) and deviant tones (50 sweeps) were averaged independently. Recordings were made with a bandpass of 0.01 - 30 Hz, and with a 500 msec sweeptime. Separate recordings were made using different interstimulus intervals (ISI): 1, 2, 3, 5, 7, and 10 seconds.

The data were analyzed by integrating the areas under each waveform generated by the standard and deviant tones. Responses to deviant tones were corrected for the mismatch attributable to a difference in sweep-number alone. The magnitude of the remaining Waveform Integral represented the animals' abilities to distinguish the deviant tones from the standard tones. The average Waveform Integrals at each ISI were statistically analyzed by Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U tests. Statistical significance was accepted at the 90% confidence level.

The normal, adult rats were 7 - 11 months old and weighed 487 ± 10 grams at the time of recording. A plot of Wave IV latencies from the auditory evoked potentials demonstrated a normal audiometric profile in this group of animals, with normal thresholds and recruitment. At each ISI, the distributions of normal, young, adult rats MMRs were skewed and sometimes with bimodal distributions. However, a comparison of mean MMRs with different ISIs (MMR-ISI profile) resembled a bell-shaped curve in which the inclination and declination represent the timecourse for the formation and degradation, respectively, of short-term, auditory memory traces. The magnitude of MMRs were smallest at 1-sec ISI, but gradually increased to a maximum mismatch at 3-sec ISI ($p < 0.05$, Mann-Whitney test, 3-sec ISI compared to both 1- and 2-sec ISI). At 5-sec ISI, the response magnitude was still robust, but declined to a minimum by 10-sec ISI.

Since MMRs are generated by the auditory association cortex, decreases in MMR magnitude are believed to be due to inaccurate or incomplete feature analysis of the auditory stimuli. Therefore, it has been argued that the declination of the MMR profile curve reflects degradation of auditory memory traces in the brain. In the present study, the animals demonstrated maximum recognition of deviant frequencies when the auditory stimuli were delivered within 3 - 5 seconds of each other. With longer ISI, auditory feature analysis became innaccurate or incomplete. These data suggest that auditory memory traces in the normal anesthetized rat were sustained for as much as 5 seconds before degradation ensued.

The aged rats were 18 months old and weighed 611 ± 7 grams at the time of recording. Audiometric analysis of the aged animals demonstrated profiles of threshold and recruitment that were not different from the young animals. However, the MMR profile of the aged animals was very different from that of their younger cohorts. Although the responses at 1-sec ISI were not different between the two groups, the aged animals showed a dramatic decrease in MMR magnitude at 2-sec ISI ($p < 0.05$).

The aged animals were obviously less efficient in performing higher order processing of auditory information than the younger animals, perhaps due to more rapid degradation of auditory memory traces. Alternatively, aging may be associated with loss of some early components of frequency analysis that result in incomplete or delayed recognition of differing tones. The observation that MMRs were decreased in the aged animals is consistent with the observations of others that working memory in rats is impaired with age.

These data represent not only the first recording of MMRs from the rat, but the first such recording in any anesthetized animal. Results from the aged animals strengthen the interpretation that MMRs recorded with a variable-ISI paradigm provide a measurement of the duration of short-term memory traces. The recording of MMRs in

anesthetized rats presents an economical model for studying the mechanisms of memory performance. This method may be of interest to a broad spectrum of Neuroscientists who are generally interested in the study of higher brain functions.

Project 15: Dietary protein and higher brain function in the rat (project coordinator: Jeffery Brock). The major objective of this study was to explore possible correlations between cortical cell morphology, cortical electrical activity, and animal behavior, using varying levels of protein in the diet as the primary manipulation. In the first part of this study, we hypothesized that rats consuming a long-term, high-protein diet present with a generalized inability to cope with stress. Male, Sprague-Dawley rats were divided into two groups (N = 7 each), which were fed diets of 50% casein (high-protein or HP group) and 20% casein (normal-protein or NP group). After consuming their respective diets ad libitum for 32 weeks, each animal was tested for 5 days (1 trial/day) using the rat swimming test of Porsolt, which measures the animal's emotional adaptation to stress. It was observed that by Day 5, the NP group demonstrated a significantly higher ($P<0.05$) immobility time than the HP group. These data suggest that the animals on the high-protein diet were less able to develop an effective strategy for coping with repeated stress.

Based upon the results of Part 1 of this study, a second experiment was performed which analyzed the short-term memory formation in the rats maintained on normal- and high-protein diets. This study tested the hypothesis that abnormal behavior in rats that consume a high-protein diet is associated with decrements in higher-order sensory information processing in the brain. In this procedure, each animal was anesthetized using alpha-chloralose and urethane (50 mg/kg and 1.5 gm/kg i.p., respectively). The body temperature was maintained at 37 $\pm$.5 °C by a heating pad placed under the animal and monitored by rectal probe. A tracheostomy was performed and the animal was permitted to breath spontaneously. The animal's head was fixed in a stereotaxic frame. Ear bar adaptors were positioned in the indention of the animal's squamosal bones. Earplug speakers were inserted into the left and right auditory meatus and secured in place with surgical tape.

Platinum needle electrodes were placed in contact with the dura mater through small holes made in the skull. A reference electrode was located at Lambda. An active electrode was located 4 mm lateral over the right parietal cortex. A ground electrode was placed over the frontal cortex. A standard tone was delivered binaurally to the animal with a frequency of 4 kHz, an intensity of 90 dB nHL, a 1 msec ramp, and a 10 msec plateau. The standard tone was delivered with a 95% probability. The deviant tone was a 6kHz frequency that was delivered with a 5% probability, but was otherwise identical to the standard tone. Recordings were made with a bandpass of 0.01 - 30 Hz, and with a 500 msec sweeptime. Electroencephalographic activity in response to the standard tones

(1000 sweeps) and deviant tones (50 sweeps) were averaged independently. Separate recordings were made using different interstimulus intervals (ISI): 1, 3, and 7 seconds. The data were analyzed by integrating the areas under each waveform generated by the standard and deviant tones. Responses to deviant tones were corrected for the mismatch attributable to a difference in sweep-number alone. The magnitude of the remaining waveform integral represents the animal's ability to distinguish the deviant tones from the standard tones at the level of the auditory association cortex, and this is called the mismatch response (MMR).

In the normal-protein diet group, the MMRs were largest at 1-sec ISI. The magnitude of MMRs declined at 3- and 7-second ISI, suggesting a degradation of short-term memory traces in a way that resulted in either incomplete or inaccurate feature analysis of the auditory stimuli within that timeframe. In the high-protein diet group, there was a high variability that prevented statistical significance compared to the NP group. However, there was a trend in the data from the HP diet animals that suggested a decrease in the magnitude of their MMRs compared to the control animals. A scatter-plot of the data comparing each animal's immobility time in the Porsolt test on Day 5, plotted against the animal's MMR recorded at 1-sec ISI suggests that there may be a correlation between an animal's response to repeated stress and its accuracy in performing analysis of auditory stimuli. Thus, animals maintained on a high-protein diet appear to be different from control animals in their ability to perform higher-order sensory information processing. Impaired development, or increased degradation, of short-term memory traces in the brain may be an important factor relating to the abnormal behavior of animals maintained on a long-term, high-protein diet.

Project 16: Dietary protein and changes in monoamine neurotransmitter levels in the rat brain (project coordinators: Jeffery Brock, Shakeel Farooqui, and Emmanuel Onaivi). This was the final data collection procedure related to the previous mission of the Neuroscience Lab, which was sponsored by Dept. of the Army Grant DAMD 17-88-Z-8023. Final analyses of the data on the effects of dietary protein on brain levels of dopamine, DOPAC, and HVA have been completed. The analyses of serotonin, 5-HIAA, norepinephrine, and epinephrine are not yet completed. The following study presents evidence that dopamine levels in the rat brain covaried with the level of protein in the diet.

Proteins and their breakdown products, the amino acids, serve as precursors for amine neurotransmitters in the brain. Studies have shown that both increasing and decreasing dietary protein levels have an effect on higher brain function in animals, although the mechanisms responsible for dietary protein-induced behavior remain unclear. Recent studies have shown that rats maintained on a chronic, high-protein diet (50% casein) demonstrated increased spontaneous locomotor activity, were more reactive to nociceptive

stimuli than rats fed either normal-protein (20% casein) or low-protein (8% casein) diets. Changes in the cerebral cortical activity of rats maintained on 50% casein were indicative of increased central catecholaminergic activity and preparatory arousal levels. Other studies have shown that the 8% casein diet resulted in a decrease in the number of dopamine D2 receptors in the rat striatum. The implication is that central dopaminergic activity may be facilitated or inhibited, respectively, by an increase or decrease in dietary-protein levels. The forebrain dopaminergic systems of the brain have been investigated extensively by neuroanatomists and behavioral pharmacologists, and continue to be of primary interest to clinicians. In the present study, the levels of dopamine and its metabolites were analyzed in the brains of the same animals for which behavioral abnormalities were reported earlier. The brain regions selected for analysis were known postsynaptic tissues for dopaminergic afferent neurons, and they represented neuroanatomically and functionally distinct dopaminergic systems in the brain: the nigrostriatal, mesolimbic, mesocortical, mesohippocampal, periventricular, incerto-hypothalamic, and descending dopaminergic systems.

Eighteen male, Sprague-Dawley rats were obtained as weanlings from Harlan Sprague-Dawley (Indianapolis, IN). The animals were divided into 3 groups (N = 6 each) and placed on one of three diets: low-protein (LP, 8% casein) ad libitum, normal-protein (NP, 20% casein) pair-fed with the LP group, and high-protein (HP, 50% casein) pair-fed with the LP group. After the animals had been on their respective diets for 8 months, all were sacrificed by decapitation and their brains were stored at -80°C.

The brains were sliced on a freezing microtome and 27 areas of the brain were collected using the punch-dissection method of Palkovits: amygdala, caudate/putamen, cerebral cortex (frontal, parietal, entrohinal), globus pallidus, hippocampal areas (dentate gyrus, subiculum), hypothalamic nuclei (anterior, lateral, medial pre-optic, posterior, suprachiasmatic nuclei), interpeduncular nucei, medial forebrain bundle, periaqueductal (central) gray area raphe nuclei (doral and medial), substantia nigra, thalamic nuclei (centromedial, inferior colliculi, medial geniculate, posterior, ventrolateral), tuberculum olfactorium, and the ventral tegmental area. The accuracy of punch location was verified by fixing the tissue sections afterward in 10% formalin, treating them with a Nissl body stain, and comparing the stained sections to Paxinos and Watson's Stereotaxic Atlas of the Rat Brain. The tissue samples were homogenized individually by sonication for 15 seconds in 0.1 M perchloric acid. The homogenates were centrifuged at 15,000 rpm for 15 minutes, then filtered through 0.45 um membranes. The pellets were reconstituted in 0.1 N NaOH for the spectrophotometric determination of protein content. Aliquots from the filtered supernatant were analyzed by reverse-phase high-performance liquid chromatography and quantitated by electrochemical detection. The average amine contents for each brain area were statistically

analyzed by single-factor analysis of variance, followed by unpaired Student's t-tests. Statistical significance was accepted at the 95% confidence level (alpha = 0.05, two-tailed test).

At the time of sacrifice, the body weights of the animals were not significantly different between the 3 diet groups (Mean ± S.E.M.): LP group, 507 ± 14; NP group, 472 ± 11; HP group, 475 ± 16 grams. The effects of the dietary protein manipulations on the contents of DA, DOPAC, and HVA in the 27 different nuclei were catagorized in terms of the dopaminergic system in the brain which they represent. Dopamine levels in the substantia nigra and caudate/putamen (which constitute most of the mesolimbic system) were significantly decreased by feeding the LP diet. Increasing dietary protein also increased dopamine content of the caudate/putamen. Dopamine levels in the ventral tegmental area and frontal cortex (which constitute the mesocortical system) were not affected by protein levels in the diet. Diminished dopamine levels observed in the medial forebrain bundle with the LP diet probably reflect the changes seen in the mesolimbic system. Monoamine oxidase activity (metabolism of DA to DOPAC) was sensitive to changes in dietary protein only in the caudate/putamen and the medial forebrain bundle, where DOPAC content was diminished by the LP diet. The activity of catechol-O-methyltransferase (metabolism of DA to HVA) was sensitive to changes in dietary protein only in the substantia nigra and frontal cortex, where HVA content diminished with the LP diet. HP diet also resulted in a decrease in HVA levels in the frontal cortex.

The values obtained from neurochemical analysis of the NP control group were similar to those published by other investigators who have examined the levels of DA, DOPAC, and HVA in the rat brain. One important aspect of this comprehensive mapping of the distribution of changes in DA levels in the brain is that the data may be interpreted from a behavioral science perspective. Heterogeneity in the distribution of DA in the brain allows for the differences in DA levels in response to manipulation to be related to the functional specialization of those brain areas. Viewing the data in this way makes it is possible to gain further insight into the physiological mechanisms that link dietary macronutrients and behavior. The distribution of changes in DA metabolism induced by the different diets suggests that specific dopaminergic systems are activated by the manipulation of dietary protein. These data demonstrate that the level of DA and its metabolites changed following dietary protein manipulation in a region-specific manner. The nigrostriatal and mesohippocampal systems were the most sensitive to changes in dietary protein, compared to the mesocortical, mesolimbic, periventricular, and descending DA systems of the brain. The incerto-hypothalamic system was remarkably insensitive to dietary protein. When changes in DA levels were apparent, DA content generally covaried with the level of protein in the diet. In conclusion, differential modulation of doraminergic activity in discrete regions of the brain may be a

mechanism by which dietary protein influences the expression of locomotor behavior in rats.

Manuscripts published/in press.

1. Charles W. Hilton, Chandan Prasad, and J. F. Wilber. Acute alterations of cyclo(His-Pro) levels after oral ingestion of glucose. Neuropeptides 15:55-59, 1990.

2. Charles W. Hilton, Chandan Prasad, F. Svec, P. Vo, and S. Reddy. Cyclo(His-Pro) in nutritional supplements. Lancet 336:1455, 1990.

3. Chandan Prasad, Francis A. Ragan, and Charles W. Hilton. Isolation of cyclo(His-Pro)-like immunoreactivity from human urine and demonstration of its immunologic, pharmacologic, and physico-

chemical identity with the synthetic peptide. Biochemistry International 21(3):425-434, 1990.

4. Cherng-Zee Chuang, Francis Avery Ragan, and Chandan Prasad. Optimization of conditions for simultaneous separation of ten tryptophan metabolites using reversed-phase high performance liquid chromatography. Journal of Chromatography 534:13-21, 1990.

5. Hiromasa Ikegami and Chandan Prasad. Neuropeptide-dopamine interactions. V. CYCL(HIS-PRO) regulation of striatal dopamine transporter complex. Peptides 11: 145-148, 1990.

6. Chandan Prasad, Charles W. Hilton, F. Svec, Emmanual S. Onaivi, and P. Vo. Could dietary proteins serve as cyclo(His-Pro) precursors? Neuropeptides 19:17-22, 1991.

7. Emmanual S. Onaivi, Stephanie Talton, and Chandan Prasad. The level of protein in diet modulates the behavioral effects of amphetamine. In: Endocrine and Nutritional Control of Basic Biological Functions. H. Lehnert, R. Murison, H. Weiner, D. Hellhammer, and J. Boyer (Eds), 1991.

8. Shakeel M. Farooqui, Jeffery W. Brock, Anwar Hamdi, and Chandan Prasad. Antibodies against synthetic peptides predicted from the nucleotide sequence of D_2 receptor recognize native dopamine receptor protein in rat striatum. Journal of Neurochemistry 57:1363-1369, 1991.

9. Jeffery W. Brock and Chandan Prasad. Motor, but not sensory, cortical potentials are amplified by high-protein diet. Physiology and Behavior 50:887-893, 1991.

10. Anwar Hamdi and Chandan Prasad. Attenuation of pulsatile changes in the density of striatal [^{3}H]GBR-12935 binding sites

during chronic ethanol consumption. Brain Research 567:71-75, 1991.

11. Chandan Prasad, Anwar Hamdi, Jeffery W. Brock, and Charles W. Hilton. Cyclo(His-Pro) and food intake. In: The Science of Food Regulation. George Bray, Ed., Louisiana State University Press, Baton Rouge, Lousiana, 1992.

12. Jeffery W. Brock, Shakeel Farooqui, Keith Ross, and Chandan Prasad. Localization of dopamine D_2 receptor protein in the rat brain using polyclonal antibody. in press, Brain Research, 1992.

13. Anwar Hamdi, Emmanuel S. Onaivi, and Chandan Prasad. A low protein-high carbohydrate diet decreases D2 dopamine receptor density in rat brain. Life Sciences 50:1529-1534, 1992.

14. Anwar Hamdi and Chandan Prasad. Bidirectional changes in striatal D2-dopamine receptor density during chronic ethanol intake. Alcohol 9:133-137, 1992.

15. Shakeel M. Farooqui, Chandan Prasad, and Massarat Ali. Production and characterization of a monoclonal antibody to dopamine D2 receptor: comparison with a polyclonal antibody to a different epitope. Biochemical and Biophysical Research Communications 184(2):661-667, 1992.

16. Masahiro Sakata and Chandan Prasad. Transient decrease in rat striatal D2 dopamine receptor mRNA level after acute haloperidol treatment. in press, Molecular Brain Research, 1992.

17. Masahiro Sakata, Shakeel M. Farooqui, and Chandan Prasad. Post-translational regulation of loss of rat striatal D2 dopamine receptor during aging. in press, Brain Research, 1992.

18. Emmanuel S. Onaivi, Jeffery W. Brock, and Chandan Prasad. Dietary protein levels alter rat behavior. in press, Nutrition Research, 1992.

19. Jeffery W. Brock and Chandan Prasad. Alterations in dendritic spine density in the rat brain associated with protein malnutrition. in press, Developmental Brain Research, 1992.

20. Shakeel M. Farooqui and Chandan Prasad. An antibody to dopamine D2 receptor inhibits dopamine antagonist and agonist binding to dopamine D2 receptor cDNA transfected mouse fibroblast cells. in press, Life Sciences, 1992.

21. Anwar Hamdi, Johnny Porter, and Chandan Prasad. Obesity and hyperphagia do not accelerate age-associated loss of striatal D_2 dopamine receptors. accepted to Brain Research, 1992.

Manuscripts in preparation.

1. Jeffery W. Brock, Keith Ross, Ashley Cowart, and Chandan Prasad. Stimulus mismatch negativity in the anesthetized rat: normative data and the effects of aging. (in preparation for Electroencephalography and Clinical Neurophysiology).

2. Jeffery W. Brock, Keith Ross, and Chandan Prasad. REM sleep deprivation and caloric intake in the rat. (in preparation for Physiology and Behavior).

3. Shakeel Farooqui, Jeffery W. Brock, Joseph LaFleur, and Chandan Prasad. Protein malnutrition increases expression of MAP2 proteins in the rat brain. (in preparation for Neuroscience Letters).

4. Shakeel Farooqui, Joseph LaFleur, and Chandan Prasad. Coupling of GTP-binding protein to dopamine D_2 receptor: presence of an epitope on the 110 kDa D_2 receptor complex recognized by antibody to amino terminal peptide of the Gi-beta. (in preparation for Journal of Neurochemistry).

5. Jeffery W. Brock, Shakeel Farooqui, Emmanuel Onaivi, and Chandan Prasad. Modulation of serotonin levels in discrete areas of the rat brain by altering dietary protein:carbohydrate ratio. (in preparation for Journal of Neurochemistry).

6. Shakeel Farooqui, Jeffery W. Brock, Emmanuel Onaivi, and Chandan Prasad. Dietary protein:carbohydrate ratio modulates central catecholamine levels in discrete areas of the rat brain. (in preparation for Journal of Neurochemistry).

7. Cherng-Zee Chuang, Francis Avery Ragan, and Chandan Prasad. Demonstration of anthranilic acid in rat brain. (in preparation for Journal of Neurochemistry).

8. Cherng-Zee Chuang, rancis Avery Ragan, and Chandan Prasad. Steady-state level of tryptophan metabolites in rat brain. (in preparation for Brain Research).

9. Emmanuel Onaivi, Stephanie Talton, and Chandan Prasad. Dietary modulation of drug action; neuroleptics and delta-9THC. (in preparation for Brain Research).

10. Emmanuel Onaivi, Shorye Payne, Jeffery W. Brock, Shakeel Farooqui, and Chandan Prasad. Nicotine and age-associated decrease in the tail-flick latency. (in preparation for Pharmacology Biochemistry and Behavior).

11. Emmanuel S. Onaivi, Shorye Payne, Jeffery W. Brock, Anwar Hamdi, Shakeel Farooqui, and Chandan Prasad. Strain differences in the effects of dietary protein on avoidance behavior in the rat. (in preparation for Physiology and Behavior).

12. Jeffery W. Brock, Keith Ross, and Chandan Prasad. Maladaptive coping patterns in the rat induced by high dietary protein:carbohydrate ratio. (in preparation for Physiology and Behavior).

13. Shakeel Farooqui, Anwar Hamdi,and Chandan Prasad. Chronic ethanol ingestion differentially regulates the activity of Gia and Go in rat striatum. (in preparation for Brain Research).

14. Jeffery W. Brock, Richard Tulley, Kerrie Munson, Keith Ross, and Chandan Prasad. Protein malnutrition and amino acid profiles in the rat brain. (in preparation for Neurochemical Research).

Abstracts.

1. Chandan Prasad and S. Eloby-Childress. Diet and urinary excretion of cyclo(His-Pro)-like immunoreactivity. Society For Neuroscience Abstracts, 1989.

2. Jeffery W. Brock, Shakeel Farooqui, and Chandan Prasad. Dopamine D_2 receptor-specific antibodies. Society For Neuroscience Abstracts 16(1):209, 1990.

3. Emmanuel S. Onaivi, Jeffery W. Brock, Anwar Hamdi, and Chandan Prasad. High-protein diet modulates dopamine- and non-dopamine mediated behaviors in rats. Society For Neuroscience Abstracts 16(2):1051, 1990.

4. Chandan Prasad. Cyclic dipeptides and neuronal function. Symposium on Endocrine and Nutritional Control of Basic Biological Functions, 1990.

5. Emmanuel S. Onaivi, Stephanie Talton, and Chandan Prasad. Level of protein in diet modulates the behavioral effects of amphetamine. Symposium on Endocrine and Nutritional Control of Basic Biological Functions, 1990.

6. Chandan Prasad. Peptidergic regulation of striatal dopamine transporter complex. International Symposium on Presynaptic Receptors and Neuronal Transporters, 1990.

7. I. Shimizu and Chandan Prasad. A correlation between the number of straital dopamine uptake sites and dopamine uptake in old, but not young rats. International Symposium on Presynaptic Receptors and Neuronal Transporters, 1990.

8. Shakeel M. Farooqui, Anwar Hamdi, Jeffery W. Brock, and Chandan Prasad. Production and characterization of antibodies to dopamine D_2 receptor using an undecapeptide corresponding to the NH_2 terminal sequence. American Federation of Clinical Research, Southern Meeting, Endocrinology Section, 1991.

9. Sheila Venugopal, Shakeel M. Farooqui, Jeffery W. Brock, and Chandan Prasad. Protein kinase C induced release of dopamine is independent of G-protein uncoupling. American Federation of Clinical Research, Southern Meeting, Metabolism Section, 1991.

10. Anwar Hamdi, Emmanuel S. Onaivi, Shakeel Farooqui, and Chandan Prasad. Level of protein in diet modulates dopamine receptor. FASEB abstracts, 1991.

11. Emmanuel S. Onaivi, Shorye Payne, Jeffery W. Brock, and Chandan Prasad. Nicotine and age-associated decrease in the tail-flick latency. 53rd Annual meeting of the Committee on Problems of Drug Dependency, 1991.

12. Jeffery W. Brock and Chandan Prasad. Motor, but not sensory, cortical potentials are amplified by high-protein diet. Third IBRO World Congress of Neuroscience, 1991.

13. Emmanuel S. Onaivi, Shorye Payne, Jeffery W. Brock, Anwar Hamdi, Shakeel Farooqui, and Chandan Prasad. The performance of Sprague-Dawley and Hooded rats in the shuttle-box avoidance paradigm is dependent on the level of protein in diet. Third IBRO World Congress of Neuroscience, 1991.

14. Masarat Ali, Jeffery W. Brock, C. Douglas Gulley, Melanie Lavergne, and Wayne V. Vedekis. Regional distribution of retinoic acid receptor alpha (RAR-alpha), -beta, and -gamma forms in rat brain. Third IBRO World Congress of Neuroscience, 1991.

15. Jeffery W. Brock, Shakeel Farooqui, Keith Ross, and Chandan Prasad. Localization of dopamine D_2 receptor protein in rat brain using polyclonal antibody. Society For Neuroscience Abstracts 17(1):415, 1991.

16. Masahiro Sakata, Shakeel Farooqui, and Chandan Prasad. Age dependent changes in dopamine D_2 receptor mRNA levels in anterior pituitary glands of Fisher 344 rats. The Endocrine Society

17. Massarat Ali, Jeffery W. Brock, C. Douglas Gulley, and Wayne V. Vedeckis. Age-related changes in regional distribution of retinoic acid receptor (RAR-beta) in rat brain. American Society for Cell Biology, 1991.

18. Jeffery W. Brock and Chandan Prasad. Alterations in dendritic spine density in the rat associated with protein malnutrition. American Federation for Clinical Research, Southern Meeting, 1992.

19. Jeffery W. Brock, Keith Ross, and Chandan Prasad. Maladaptive coping patterns in the rat induced by high dietary protein:carbohydrate ratio. Journal of Cellular Biochemistry suppl. 16B:261, 1992.

20. Shakeel M. Farooqui, Jeffery W. Brock, Joseph W. Lafleur, and Chandan Prasad. Region specific modulation of rat brain dopamine levels by long term changes in the dietary protein. Journal of Cellular Biochemistry suppl. 16B:264, 1992.

21. Anwar Hamdi, Emmanuel S. Onaivi, and Chandan Prasad. Macronutrients modification alters D2 dopamine receptor properties in rat brain. Journal of Cellular Biochemistry suppl. 16B:264, 1992.

22. Shakeel M. Farooqui, Chandan Prasad, and Massarat Ali. Production and characterization of a monoclonal antibody to dopamine D2 receptor. The Endocrine Society, 1992.

23. Jeffery W. Brock, Shakeel Farooqui, Emmanuel S. Onaivi, Anwar Hamdi, and Chandan Prasad. Dietary protein modulates dopamine levels in the rat brain. submitted to Society for Neuroscience, 1992 meeting.

24. Shakeel Farooqui and Chandan Prasad. Retinoic acid mediated induction of dopamine D2 receptor in human SHSY-5Y neuroblastoma cells. submitted to Society for Neuroscience, 1992 meeting.

25. Amwar Hamdi, Jeffery W. Brock, Keith Ross, Shorye Payne, and Chandan Prasad. REM sleep deprivation and dopamine receptor binding

in rat striatum. submitted to Society for Neuroscience, 1992 meeting.

IV. Fort Polk Heart Smart Project

Introduction

The Fort Polk Heart Smart Project was funded until July 28, 1991. In the appendix is correspondence from Colonel E. Wayne Askew requesting an individual Final Report for this project. On August 15, 1992 an interim report was made for the project. That report is presented below.

During the mid-1980's, the U.S. Congress felt the need to examine issues of nutrition and general health status among military personnel and their dependents. This resulted in a congressional mandate to the U.S. Army in 1987 to devote funds to research in these issues. A portion of this funding went to a team of researchers under Dr. Gerald Berenson. Tasked to gather descriptive data and to implement a health promotion program, the team chose Fort Polk, Louisiana as its research site.

Fort Polk is the current home of the 5th Mechanized Division and has a contingent of about 15,000 active duty personnel (see Table 1, in Appendix). It represents about 8,000 - 9,000 military

families and about 6,000 - 8,000 child dependents. As such, Fort Polk was of sufficient size to conduct the required research.

Accomplishments

Over the course of two years of data collection (Summer, 1989 - Summer, 1991) the Fort Polk Heart Smart Project accomplished the following:

1. Nutritional, Lifestyle, and Physical Activity Assessments of 200 Military Wives

2. Cardiovascular Disease (CVD) Risk Factor Screening of over 700 Soldiers, Their Spouses, and Their Children

3. The Development of Health Promotion Materials for Military Families

4. The Implementation of a Health Promotion Program in about 70 Military Families

Goals and Project Design

As implied above, four basic goals were developed for research at Fort Polk. These were:

To describe typical eating, activity, and other lifestyle traits of military dependents.

To develop data on levels of CVD risk factors in military families.

To develop appropriate materials and techniques for military family health promotion programs.

To test such materials on military families.

Fort Polk Substudies

Research at Fort Polk was divided into three projects:

Project 1 - Assessment of Dietary Intake and Physical Activity in Military Wives

A sample of 200 wives intact military families underwent a battery of nutritional and other assessments to establish routine eating, activity, and other behavioral patterns (Table 2 in Appendix). A CVD risk factor screening was also offered.

Project 2 - Cardiovascular Risk Assessment of Military Families

A sample of about 125 intact military families received a CVD risk factor screening including blood pressure, anthropometric, lipid, and lifestyle questionnaire determinations (Table 3 in appendix).

Project 3 - Family Health Promotion

A program for family health promotion, including prudent eating, exercise, stress and smoking reduction was developed and implemented on about 70 intact military families (Table 4 in Appendix). Participants received CVD risk screenings, dietary and activity recall, health history, and psycho-social questionnaire evaluations on a pre- and post-implementation basis.

Results

Nutritional, demographic, socio-economic (SES), and sociological data on Project 1 wives and their families are included in Attachment 2-A, Appendix.

Demographic and SES data are presented first. Race distribution data show the sample to be about 2/3 white, 1/5 black, less than 1/10 Hispanic or other. Husbands' rank shows whites to predominate at the E-1 to E-4 and also the 0-1 to 0-4 level, while E-5 to E-9 and WO-1 and WO-2 are slightly over-represented among blacks. About 80% of the sample has a high school diploma or some college education. Median family size was 4. About 10% of the wives participated in the federal Woman, Infant, Child (WIC) program, while 1.5% received food stamps. Other data are available on number of cars and television sets owned per family.

Dietary information begins with family meal and snacking patterns. Families in this sample typically eat one meal per day together and wives enjoy 1-3 episodes of between-meal snacking on a typical day. Families also usually eat four or more meals per week while watching television.

Monthly food consumption practices show milk, bread, and margarine to be the most frequently eaten food items with organ meats and veal to be most infrequently used. Other frequently consumed items include ketchup, chips, candy, cheese, eggs, hot dogs, poultry, and cereal. After margarine, mayonnaise and cooking oil are the most frequent sources of fat and oil.

Monthly grocery expenditures range between $100 and $250 for about 2/3 of the families. Less than 10% of this group spends $350 or more while less than 5% spends less than $100. About half of these families spend $23 - $50 per month eating out.

Most families (about 60%) eat fried food regularly. Whole milk is somewhat preferred over 2% and skim.

About 2/3 of the Project 1 women report desiring to lose weight. Most want to lose between 5 and 20 pounds, but about 20% wish to lose in excess of 30 pounds.

The balance of nutrition data depict nutrient intake, adherence to recommended daily allowances (RDA), and comparability of Fort Polk results to other surveys. Overall, these women consume between about 1800 and 2000 kcals per day, with about 35% of kcals coming from fat (12-13% from saturated fat). Black women report the lowest daily caloric intake (1771 kcals). Wives of enlisted men report high intakes (2101 kcals).

Generally, the women in this sample report intake between 40 and 70% of RDA for most vitamins and minerals. Those figures are slightly better for the B-complex vitamins.

Comparisons with other surveys show Fort Polk women to be intermediate in caloric consumption and low in percent of calories from fat. Reported carbohydrate intake is relatively high at Fort Polk with cholesterol intake relatively low.

CVD Risk Factor Data

(Note: This portion of the final report replicates the findings presented in the 1990-1991 annual report. In the interest of keeping attachment material to a minimum, the appropriate tables and figures are to be found as Attachments A-C in the 1991 Annual Report and may be obtained by request.

Attachment A of the 1991 Annual Report contains summary figures and tables for the CV risk factor and lifestyle questionnaire evaluations completed on Fort Polk personnel and their dependents. Representative figures and tables from Attachment A of the 1991 Annual Report are appended and are designated Attachment A-1 and A-2. Table A-1 outlines the age, race, and sex characteristics of this group. 703 individuals participated in CV risk screening ending in January 1991. Adult females predominated reflecting their sole participation in Project 1. In the subsequent descriptions, comparisons are made with Bogalusa Heart Study norms on an age-appropriate basis. Racial comparisons are limited to black/white contrasts.

Attachment A of the 1991 Annual Report presents anthropometric and body composition results for this group. Overall, the Fort Polk sample shows little racial difference in height or in comparison with the Bogalusa population. Weight shows systematic contrasts within Fort Polk females, with adult blacks being systematically heavier. There is also a tendency for the Fort Polk adults to be heavier than their Bogalusa counterparts.

Measures of obesity show typical racial contrasts. By all gauges, skinfold and Quetelet (body mass) index (wt/ht^2) black

women exceed white ones. White men demonstrate thicker skinfold measures than black men, but similar Quetelet figures. Upper quartile of Quetelet index (greater than 27.0) includes 40-50% of whites at Fort Polk and 40-70% of blacks depending on age and sex.

Blood pressure data are presented in Attachment A of the 1991 Annual Report. As is seen nationally, black adults at Fort Polk show somewhat higher levels of both systolic and diastolic measures than whites. Pressures, however, are systematically lower when compared to Bogalusa norms.

Lipid data are presented in Attachment A of the 1991 Annual Report. Generally, black men exhibit higher levels of both total cholesterol and low-density lipoprotein (LDL) while no clear trend is evident in women. In addition, black men at Fort Polk show higher values relative to Bogalusa norms. High-density lipoprotein (HDL) levels are higher in black males than in white ones, while no clear pattern emerges among females at Fort Polk. Comparisons with Bogalusa results show little contrast among whites but do show consistently higher levels in Bogalusa blacks. Very-low-density lipoprotein tends to be systematically higher in whites of both sexes.

Approximately 40-50% of Fort Polk men of both races exceed NCEP guidelines for moderate elevation of total cholesterol (greater than 200 mg/dl). Black men additionally show 10-20% with high levels of cholesterol (greater than 239 mg/dl). Women fall in the moderately elevated range about 30-40% of the time with, typically, 5-10% falling in the high grouping. All race-sex groups, except white males, at Fort Polk exceed their Bogalusa counterparts for excessive levels of cholesterol. LDL results mirror those of total cholesterol, as expected.

A figure of 34 mg/dl or less of HDL is a potential risk category for heart disease. Fort Polk screenees show little tendency in this direction, with fewer than 5% of the sample of either sex, overall, falling into this assignment. By comparison, Bogalusa residents fall below 35 mg/dl at a rate 2-4 times greater than Fort Polk adults.

Blood chemistry data are presented in the Annual Report 1990-91. Overall, blacks tend to exceed whites in levels of all proteins and related molecules, LDH, calcium, phosphorus, and creatinine. Whites exceed blacks in levels of urea and uric acid. No consistent trends are evident in other measures.

Hematology results reveal, as is usually seen, that whites exceed blacks in levels of hemoglobin, hematocrit, and white blood cell (WBC) count.

The final section of Attachment A of the 1991 Annual Report is amended as Attachment A-2 and presents data on lifestyle

influencing variables including smoking behavior, alcohol consumption, socio-economic (SES) indicators (military rank, educational level, etc.), and family size. Smoking behavior statistics indicate that about between 36 and 47% of adult males and about 30% of adult females classify themselves as smokers. All race/sex groups except white males, who are much higher, approximate National Health Survey norms in this respect. Alcohol consumption is reported on a regular basis by between 40 and 70% of adult respondents. Blacks report a higher prevalence of drinking than other races. Overall reported alcohol consumption tends to be higher at Fort Polk though is typical of the military world-wide and is consistent across ranks in men.

Family Health Promotion

The Family Health Promotion (Project 3) component of the Fort Polk Heart Smart Project was initiated with a pilot study on 6 families during the summer of 1990. The study began on a full scale in September of 1990. Three cycles of program administration occurred between that time and July 1991. Approximately 70 families, comprising about 225 individuals, participated.

The practical goal of this sub-study was to develop a self-contained health education and promotion model suitable for military families and to deliver this model to test for acceptability, efficacy, and its effect on CVD risk factor levels and behaviors.

Attachment B of the 1991 Annual Report contains the resulting Health Promotion Manual. It is available on request. It comprises a statement on background, rationale, and theoretical base and then proceeds through the mechanisms of delivering such a program at a military installation.

The actual program consisted of a CVD risk factor screening and a battery of health-related questionnaires delivered on both a pre- and post-test basis and a set of educational sessions deliverable in a 8-12 week time frame. The manual describes session topics, agendas, and ancillary activities as well as incentive and maintenance programs. Calendars of actual sessions are also presented.

Attachment C of the 1991 Annual Report contains the complete battery of Project 3 evaluations and is available on request. Included are: standards and normative data, CVD risk factor screening data forms, lifestyle questionnaires, nutritional assessments, and process/program evaluations. Ancillary subject communication forms (screening feedback, consent letters, etc.) are included.

The data collection phase for Project 3 ended on July 25, 1991. Data are entering the final edit stage and, excepting

dietary recall information, will be available for analysis in August, 1991. Nutritional assessments require product and menu research and are scheduled for final analysis during the fall of 1991.

Conclusions

Research at Fort Polk has served to increase the descriptive database for CVD and related biomedical traits in service personnel. In addition, it has extended these observations to military dependents. Such information will prove valuable for future policy formation.

Comparisons with various standards demonstrate overall similarity of military families with American society as a whole. This indicates general nutritional and at least short-term, health status adequacy. It also implies similar susceptibility to long-term chronic or other conditions such as heart disease, diabetes, and certain cancers. In addition military families face environmental stresses unusual in civilian settings. Families must contend with regular absences of parents, who may spend more than six months of a year off-post attending regular military training functions. Moreover, as we saw twice during our research period, war may unpredictability remove family members for long periods with no certainty of return. The emotional fall-out from these responsibilities can be enormous.

These considerations make military families prime candidates for general health and wellness promotion programs. Preventive medicine both on the strictly biological and emotional levels offers great potential savings. These should be evident in reduced costs for medical attention and improved productivity in service personnel.

The Fort Polk Heart Smart Project has developed a model for family health promotion which addresses those issues. It is in manual form, transferable to a number of settings. It may be administered via a variety of military institutions; military hospitals, health promotion committees, volunteer groups, etc. It is well received by families and can be modified to serve current needs. Its adoption by the Army would well augment current health promotion activities available at military installations.

V. Menu Modification Study

Introduction and Background

Since 1985, nutrition initiatives have been introduced into the Armed Forces Recipe Service, the Army Master Menu and the Army Food Service Program to provide soldiers with diets lower in sodium, fat, and cholesterol. The Military Nutrition Division of the United States Army Research Institute of Environmental Medicine

(USARIEM) has conducted assessments of soldiers' nutrient intakes. These studies resulted in the following nutrition related recommendations: continue revision of the Armed Forces Recipe File to reduce sodium in recipes, continue to decrease the percentage of calories obtained from fat to 35% or less of total calories, and provide soldiers low cholesterol, low fat alternatives to eggs, and evaluate the acceptability and impact of using this approach to moderate soldiers' cholesterol intakes.

The Menu Modification Project incorporates modification of two weeks of Army garrison menus to meet the nutrition targets specified by the Army. The purpose of the Menu Modification Project is to provide healthful, nutritious menu selections which moderate soldier's sodium, fat, and cholesterol intakes.

Progress

The Army Menu Modification Project began in January, 1990. Human subjects Institutional Review Board approval was obtained from the Louisiana State University, Baton Rouge campus Committee on the Use of Humans and Animals as Research Subjects, the Human Use Review and Regulatory Affairs Office of the Surgeon General, U.S. Army, and the LSU Medical Center Institutional Review Board.

During the first year of the project, three part-time student workers were hired and trained to prepare menu items for taste panel testing. Recruitment, selection, orientation and training of nine volunteer taste panel participants was completed. A graduate assistant was hired to monitor preparation, service, and evaluation of approved modified menu items in the LSU athletes' dining facility. A total of 69 items were prepared and evaluated by the taste panel. Eighteen food formulations were prepared in quantity, served and evaluated for acceptability by the athletes in Broussard Cafeteria. Taste panel and athletes' scores can be found in Appendix V of the 1990 Annual Report.

Analysis of Army and modified recipes was carried out using The Extended Table of Nutrient Values (ETNV). Initially, Army recipes were modified and analyzed. Later, a full day's menu as served by the Army was modified and comparisons made between the regular and modified menus. While striking differences were not seen in initial experiments, trends were noted. Fat was lowered from 42.5 to 39% of calories. Reduction of eggs at breakfast resulted in a decrease of 814 mg to 450 mg of cholesterol. Unfortunately, sodium content of the modified menu was not lowered. Comprehensive analyses of the initial experiments are included in Appendix VI of the 1990 Annual Report.

During the second year of the project, sixteen recipes were prepared in quantity and served to athletes dining in Broussard Cafeteria on the Louisiana State University campus. Students rated the items for acceptability. Quantity preparation of recipes was

conducted in Broussard Cafeteria. The results of the acceptability ratings are also found in the Ninth Quarterly Report.

During the Tenth Quarter, five days of menus were modified and analyzed using the Extended Table of Nutrient Values (ETNV). The data was presented to Army officials at the time of their December 13, 1990 visit to the Pennington Center. The following conclusions were drawn from the data presented (see Tenth Quarterly Report):

1. Modifications resulted in a decrease in fat from 40% to 36% of calories and in saturated fat from 12% to 10% of calories.

2. Carbohydrate represented 48% of calories in the modified menu and 45% in the regular menu.

3. In terms of calories, protein was only 1% higher in the modified menu (16% vs. 15%).

4. There was a significant reduction in calories (12%) from a mean of 3500 to 3080 per day.

5. Cholesterol was significantly reduced (36%) from a mean of 720 mg to 462 mg per day. A comparison of breakfast menus also showed a significant reduction in cholesterol.

In January, 1991, a meeting was held at USARIEM to review the progress and plans for the Menu Modification Project. The results of this meeting were as follows:

1. The original two week menu cycle from the Army 1989 Master Menu would be replaced with a more current version from the 1991 Army Master Menu.

2. The fat content of the menu revision would be modified from 35% of total kilocalories from fat to more closely approach 30% of total kilocalories from fat. Individual recipe items would be reformulated as needed to more closely approach the lowered level of fat.

3. A nutrient analysis of the two weeks of Army menus would be calculated using all selections offered on the menu, an individual analysis of each menu items and a two week analysis of the average both the regular and modified menus.

4. Acceptability testing by student athletes would be discontinued due to lack of an adequate number of participants.

5. Quantities for 100 portions would be checked.

In February 1991, Dr. Catherine Champagne (Johnson) presented a paper entitled "Computer Analysis of Army Recipes and Menus Using the Extended Table of Nutrient Values (ETNV)" at the annual meeting

of the Southern Association of Agricultural Scientists in Fort Worth, Texas. An abstract of this paper can be found in the Ninth Quarterly Report.

Coding and analysis of Army and modified menus was continued. Two hundred and sixty recipes were submitted to Nutrient Data Systems at the Pennington Biomedical Research Center for analysis using the Extended Table of Nutrient Values. Quantity recipe testing was continued until the end of the spring 1991 academic semester in a student cafeteria on the Louisiana State University campus.

The analyses of 101 modified and regular Army recipes can be found in the appendix of the Twelfth Quarterly Report.

During summer of 1991, a week of Army menus was analyzed using the Extended Table of Nutrient Values for comparative purposes. The menus analyzed and results of the analysis were included in the Quarterly Report of August 1, 1991-October 31, 1991 (Thirteenth Quarterly Report). These data were presented to Army officials and the Committee on Military Nutrition during their September 18-20, 1991 visit to the Pennington Center. In addition, Dr. Catherine Champagne presented the attached data in an Army briefing on Monday, September 23, 1991.

From November 1, 1991-January 31, 1992, breakfast menu items were prepared in batches of 25 in the LSU student cafeteria. The LSU Food Service was unable to prepare the breakfast menu items in batches of 100 so the plan was for this to be completed in the Pennington Biomedical Research Center Quantity Preparation kitchen the following quarter. Quantity preparation of other menu items continued in the student cafeteria on the Louisiana State University campus. The recipes prepared included Italian Meat Sandwich, Italian Vegetable Bake, Beef and Spinach Pita Sandwiches, Chicken and Spinach Salad.

Twenty-five completed recipes were submitted to the Army for review. Nutritional analysis of recipes was carried out using the Extended Table of Nutrient Values (ETNV).

Data from the 1991 studies were presented at the 89th Annual Meeting of the Southern Association of Agricultural Scientists, Food Science and Human Nutrition Section, February 2-5, 1992, in Lexington, Kentucky. The title of the presentation was "Nutritional Analysis of Seven Days of Modified vs. Regular Army Menus Using the Extended Table of Nutrient Values (ETNV)." The abstract is included in the appendix of the Quarterly Report of February 1-April 30, 1992 (Fourteenth Quarterly Report).

Drs. Ryan and Champagne attended the Research and Development Associates for Military Food and Packaging Systems, Inc.'s (R & DA) 46th Annual Spring Meeting and Exposition held March 23-25, 1992 in

Arlington, Virginia. Further details of the meeting are included in the Quarterly Report of February 1-April 30, 1992.

During the February 1, 1992 - April 30, 1992 quarter, initial plans were formulated for changes to the menu modification project. The main focus of the project was in the area of implementation of the project at an actual U.S. garrison such as the facility at Fort Polk, Louisiana. As part of this plan, a culinary research associate, Kevin Gilley, was hired.

Catherine Champagne and Kevin Gilley, accompanied by MAJ Cecilia Thomas, visited the Ft. Polk Installation on June 1, 1992 to plan future implementation of the project at that facility. A copy of the trip report can be found in the Quarterly Report of May 1-July 27, 1992 (Fifteenth Quarterly Report).

On June 3, 1992, the Committee on Military Nutrition Research were briefed on the plans for the new Menu Modification Project to be funded from another grant for 1992-93. A handout was presented to the Committee reviewing the past progress of this research and outlining future plans (for complete report refer to Quarterly Report of May 1-July 27, 1992).

The goals include keeping kilocalorie content of menus similar to current menus while reducing fat content, reducing fat content of menus to 30% of kilocalorie content in keeeping with the Army's proposed updated nutrition standards, reducing cholesterol content of menus to no more than 300 mg/day, and emphasizing efforts to reduce sodium content of recipes/menus, which has been the most difficult task during previous work.

Committee members made several suggestions on methodology for doing garrison dining facility studies using the newly developed recipes/menus:

1) Consider intermingling new menu days into already existing menu when the study is done rather than study one full week of existing menu then immediately studying a full week of totally new menus (novelty of new menus will confound results).

2) Running the new menus several times in menu cycle then doing study (again to reduce the novelty impact of new menus)

3) Doing periodic acceptance tests of recipes at Ft. Polk or have Ft. Polk personnel periodically come to PBRC to test acceptance of recipes.

Catherine Champagne and Kevin Gilley traveled to Ft. Lee, Va and Natick, Ma to meet with Army recipe developers and menu planners and tour the Quartermster School (ACES). A trip report for these visits is contained in the Quarterly Report of May 1-July 27, 1992.

Conclusions

To achieve the nutrition-related recommendations set by USARIEM, it is apparent that further work needs to be done in the area of menu modification of Army menus. Initial data from the analysis of regular Army menus revealed the fact that fat, cholesterol and sodium are significantly higher than desirable. From our menu alterations, it was evident that improving breakfast menus led to the most significant reduction in fat and cholesterol in soldiers' diets. More work in modifying other meals will help to achieve our objectives of reducing total fat, sodium, and cholesterol. While it is evident that reducing sodium may be a more difficult task, additional work should be devoted to this project.

The findings of this project will be carried on into a new project. The main focus of the newly redesigned Menu Modification Project is implementation of the project at an actual U.S. garrison such as the facility at Fort Polk, Louisiana. Ethnic dishes, breakfast dishes, and other main and side dishes will be developed for potential incorporation into the Army Master Menu. The need for more ethnic recipes and menus was reemphasized at the Ft. Lee and Natick visits. A benchtop panel of 20 persons with food experience will be utilized to provide guidance in acceptability testing. Overall acceptability testing will consist of a larger panel of 36 members. The nine-point hedonic scale currently used by the military would be the instrument used to test product acceptability.

Once the study is outlined for implementation at Ft. Polk, acceptability will be conducted through a cooperative arrangement with Louisiana Tech University in view of their closer proximity to the facility as compared to Pennington. Graduate students from Louisiana Tech will develop ancillary studies for individual research projects. Catherine Champagne will continue nutritional analysis of modified menus using the Extended Table of Nutrient Values to present data to Army officials and at professional scientific meetings.

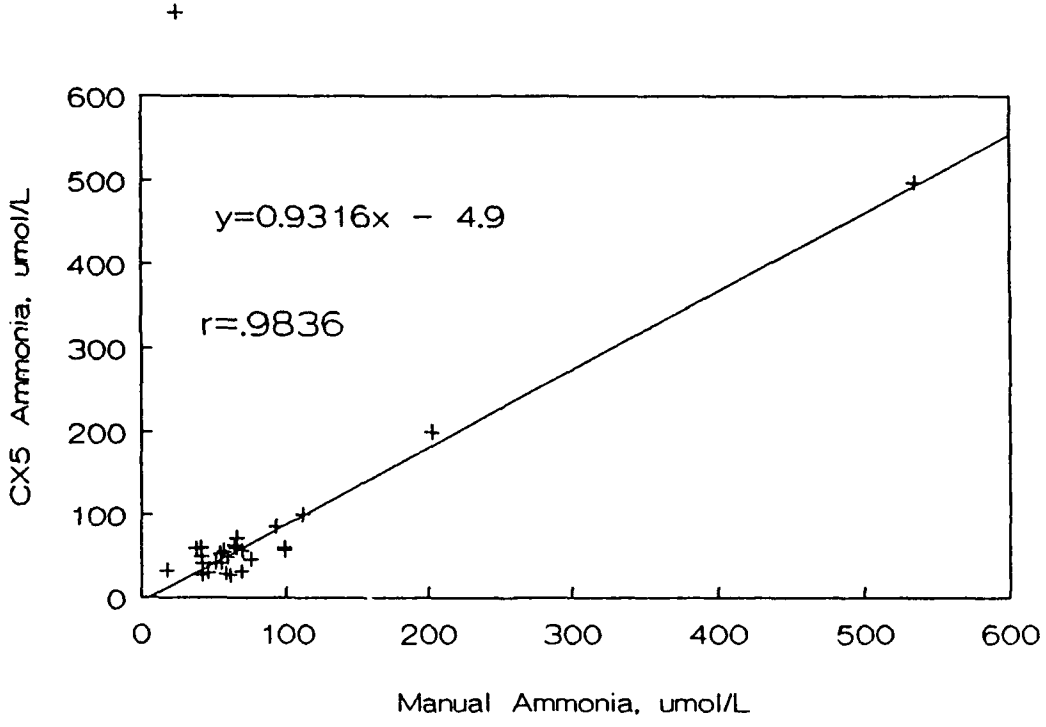
From previous research and planned future directions for the project, continuation of the Menu Modification Project will enable the military to enhance the Armed Forces Recipe File with versatile, healthy, and innovative new items. The focus on developing recipes meeting breakfast needs, as well as including ethnic dishes, addresses needs expressed by administrators both at the Quartermaster School and Center and at Natick.

APPENDICES

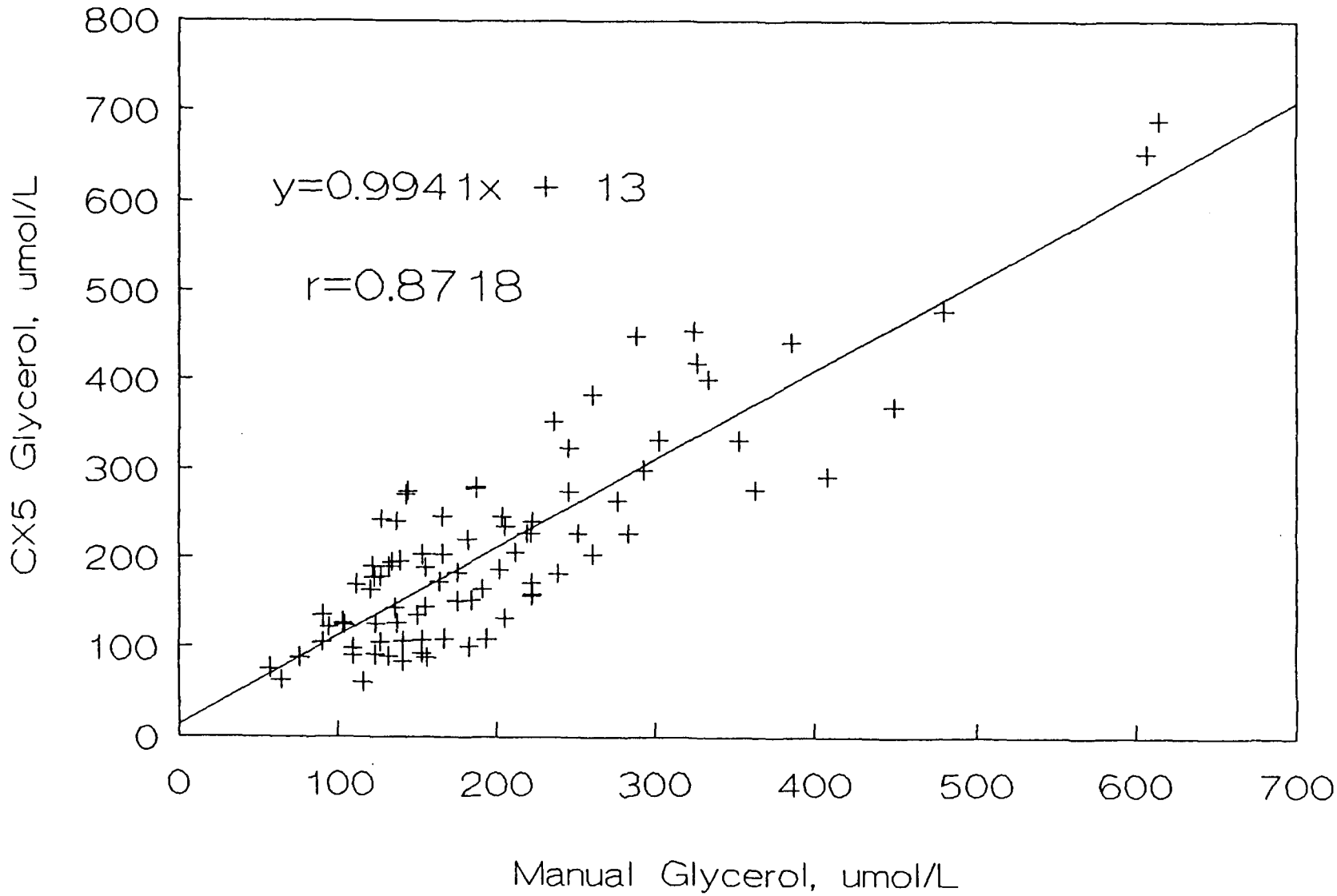
APPENDIX

Clinical Research Laboratory

Ammonia Correlation
CX5 vs Manual

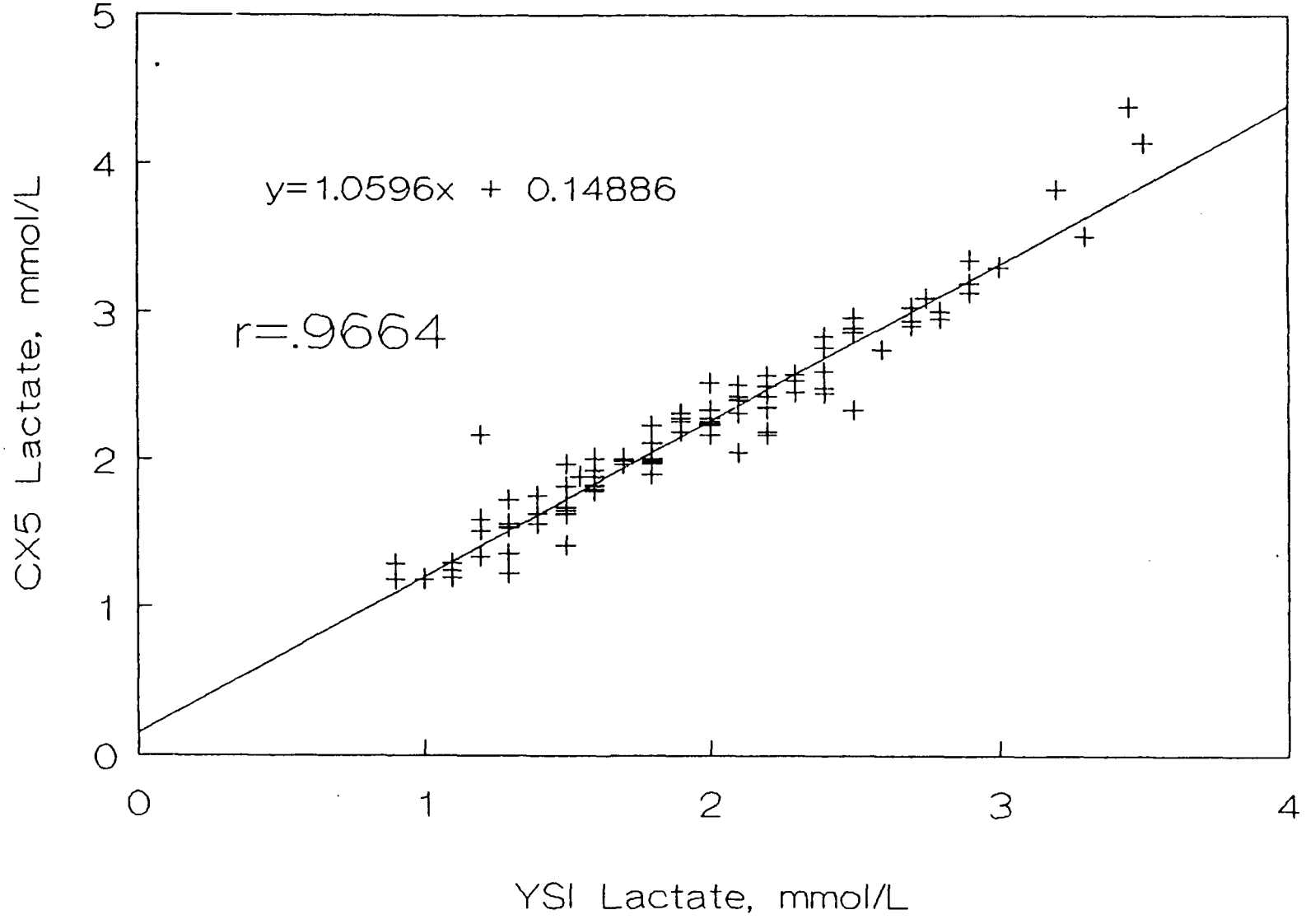


Glycerol Correlation
CX5 vs Manual



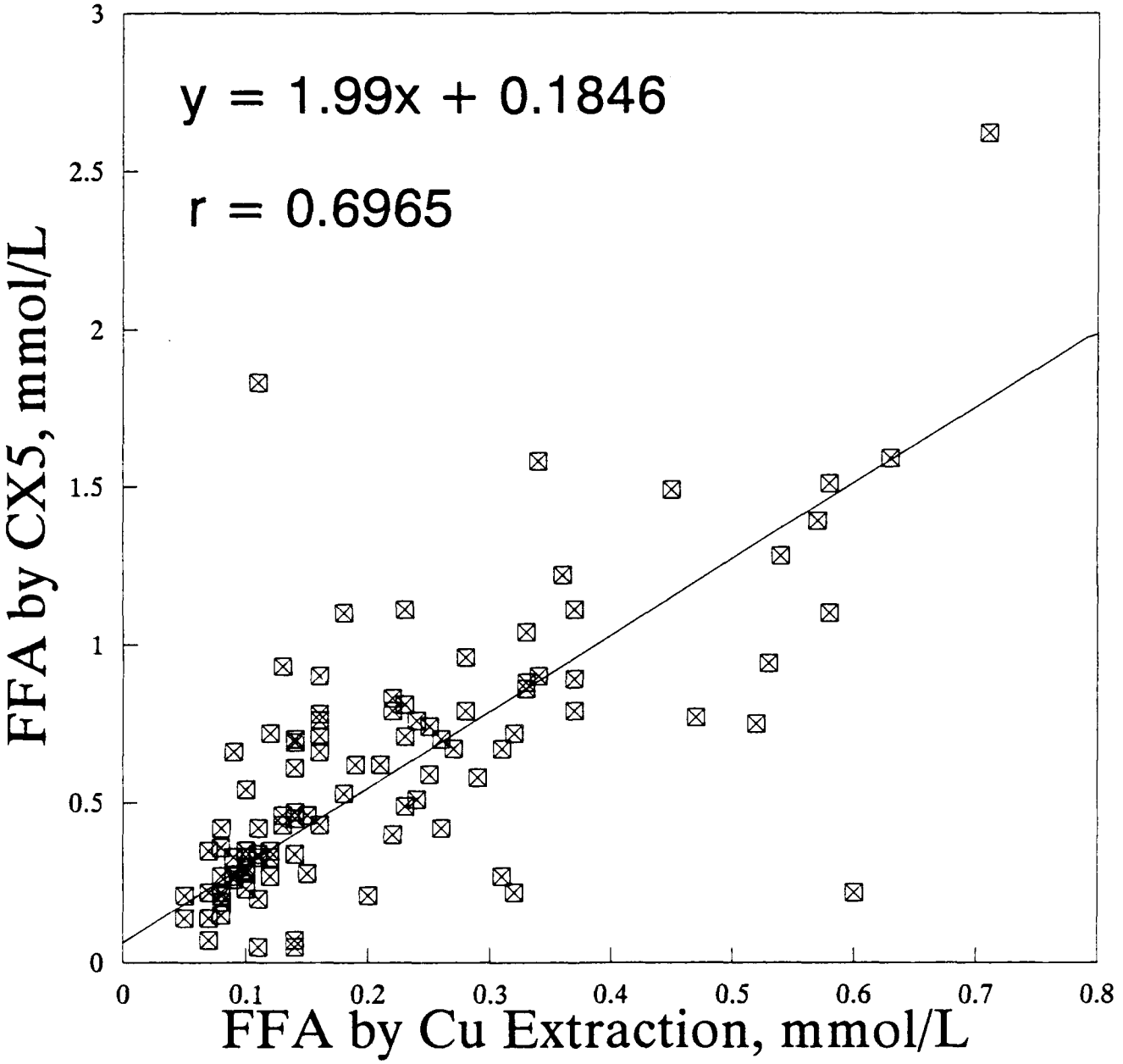
Lactate Correlation

CX5 vs YSI Lactate Analyzer

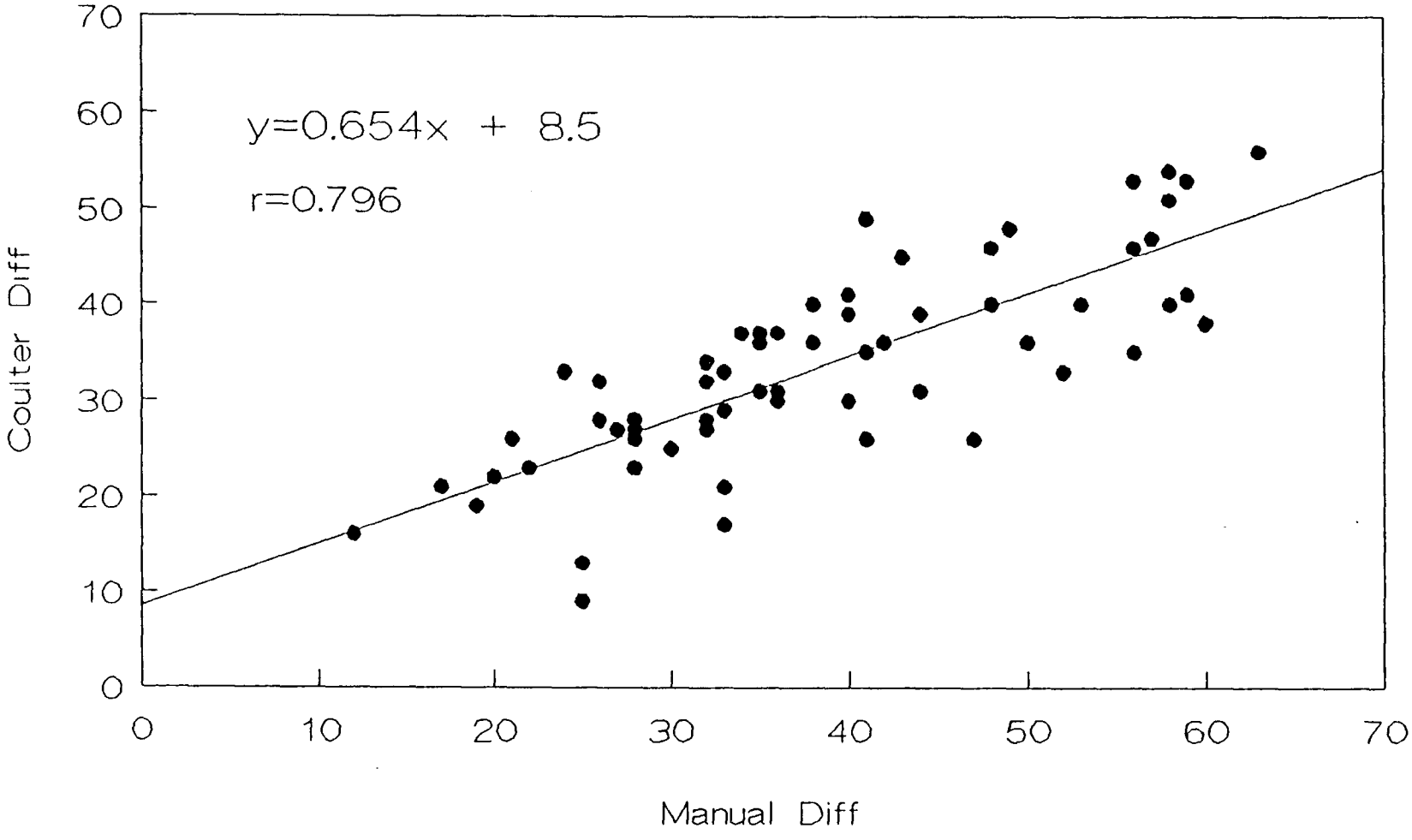


FFA Correlation

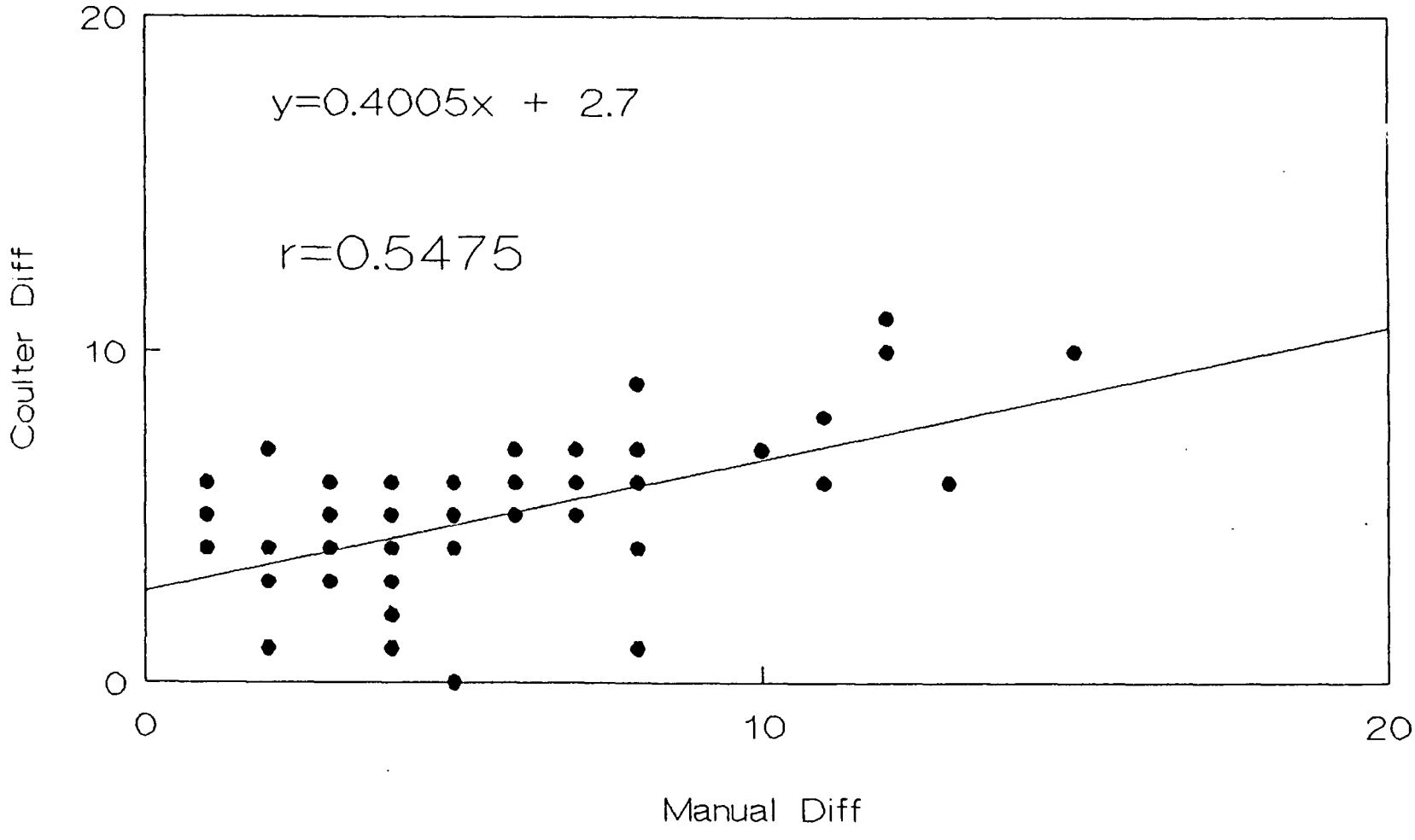
CX5 vs Cu Extraction



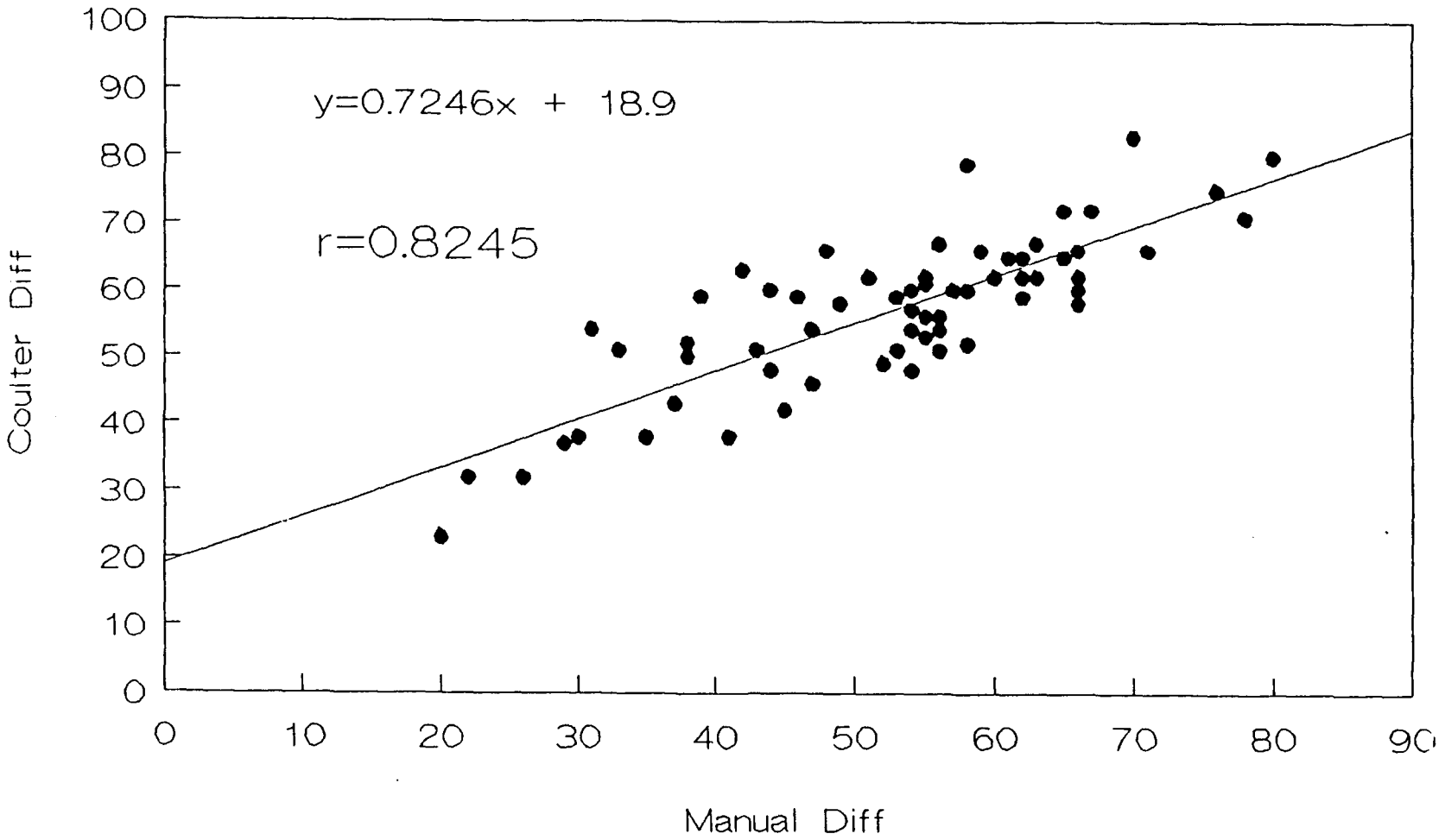
Coulter STKS
Lymphocytes



Coulter STKS
Monocytes



Coulter STKS
Neutrophils

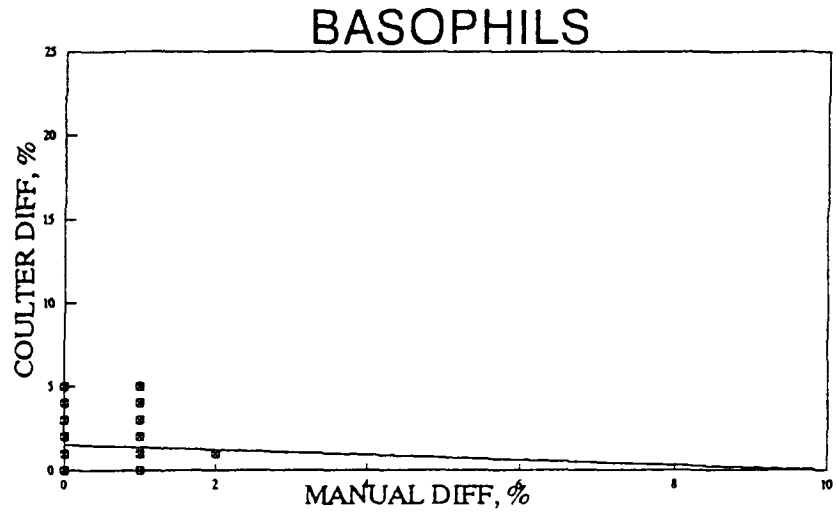


BASO

COULTER	MANUAL
3	1
4	0
1	0
0	1
1	0
1	0
1	2
0	1
1	0
1	0
1	0
2	0
1	0
1	0
3	0
0	1
2	0
1	1
2	0
1	0
1	0
1	0
1	0
1	0
1	0
1	0
0	1
0	1
3	0
1	1
2	0
1	0
1	0
1	0
1	0
1	1
2	0
5	0
1	0
1	1
2	0
2	0
1	2
1	1
1	0
2	0
2	1
3	0
1	2
1	0
1	1
2	1
1	0
1	1
1	1
4	1
1	0
1	1
2	0
2	0
1	0
1	0
1	1
1	1
1	1

Constant	Regression Output:	1.53550296	
Std Err of Y Est		1.00383496	
R Squared		0.02049448	0.14315892
No. of Observations		79	
Degrees of Freedom		77	
X Coefficient(s)		−0.2514793	
Std Err of Coef.		0.19812619	

0	1.53550296
2	1.03254438



BASO	
COULTER	MANUAL
5	1
1	1
1	0
1	1
1	1
3	1
1	0
2	1
3	0
1	1
1	1
0	0
1	1
1	1

EOS

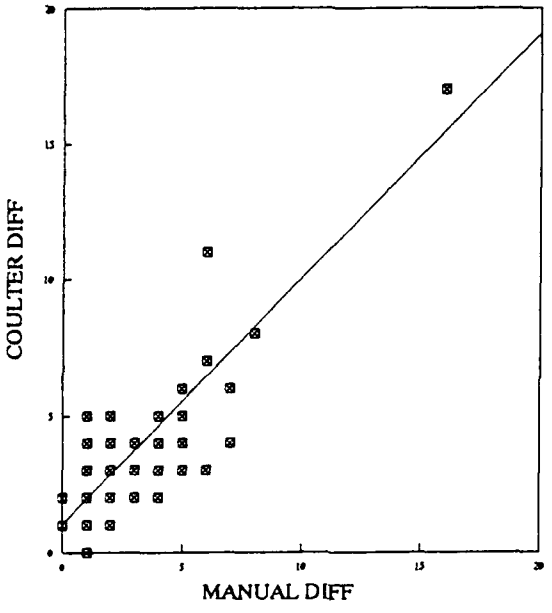
COULTER	MANUAL
1	2
6	5
1	1
8	8
4	3
1	1
3	1
3	6
2	2
4	5
3	2
5	4
2	1
2	2
3	3
17	16
4	3
4	2
3	1
4	1
2	1
4	5
3	4
2	1
1	1
1	2
1	0
4	4
3	3
5	5
5	2
2	0
3	2
1	1
3	4
0	1
2	4
5	1
3	2
4	5
3	4
2	0
2	1
4	3
1	0
2	2
3	4
2	1
2	1
3	1
3	4
2	2
1	2
1	0
1	2
4	7
4	4
2	1
2	3
2	3
2	2
3	2
2	0
4	4
2	1

Regression Output:

Constant	0.916	
Std Err of Y Est	1.3221	
R Squared	0.6568	0.8104
No. of Observations	91	
Degrees of Freedom	89	
X Coefficient(s)	0.81	
Std Err of Coef.	0.0621	

0	0.916
10	9.0161

EOSINOPHILS, %



EOS

COULTER	MANUAL
3	2
1	0
6	7
2	2
7	6
2	1
2	2
4	4
1	2
2	3
5	5
1	2
5	1
11	6
2	2
3	3
4	3
3	5
3	2
3	2
3	2
1	2
2	2
4	3
4	2
4	2

APPENDIX

Fort Polk Heart Smart Project

DEPARTMENT OF THE ARMY
US ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE
NATICK, MASSACHUSETTS 01760-5007

July 20, 1992

Military Nutrition Division

Donna H. Ryan, M.D.
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, Louisiana 70808

Dear Dr. Ryan:

Pursuant to our discussion of the completion of work on grant #DAMD 17-88-Z-8023, I would like to request a final report on the Fort Polk Heart Smart project. At the time of the on-site review, September 19, 1991, Dr. Berenson had not finished analyzing and summarizing all of his data from the final phase of the project.

Since this project was one of the more extensive efforts of this grant and is of considerable importance to the Army, we would like to have a report detailing goals, methods, results, and recommendations. While this information may be eventually published in several journal articles, it would be very useful to us to have a single integrated report of the total project.

Thank you for your assistance.

Sincerely,

Eldon W. Askew, Ph.D.
Colonel, U.S. Army
Grant Officer Representative

Copy Furnished:

Colonel Schnakenberg, Director, Army Systems Hazards,
U.S. Army Medical Research and Development Command

Pennington Biomedical Research Center
LOUISIANA STATE UNIVERSITY

July 28, 1992

COPY

Colonel Askew, Ph.D.
Chief, Military Nutrition Division
U.S. Army Research Institute of Environmental Medicine
Natick, Massachusetts 01760-5007

Dear Colonel Askew:

As you requested in your letter July 20, 1992 I will ask Dr. Gerald Berenson to provide a final report on the Fort Polk Heart Smart Project.

In prior correspondence (copies attached) I have asked for submission of the Fort Polk Heart Smart Project Report as part of the Final Report for Grant #DAMD17-88-Z-8023. However, I will revise this request and ask that Dr. Berenson submit a project report to be provided separately.

Please contact me if I can provide further information or assistance.

Sincerely,

Donna H. Ryan, M.D.
Associate Executive Director

mcl

6400 Perkins Road, Baton Rouge, Louisiana 70808-4124 ♦ Phone: (504) 765-2500, Fax: (504) 765-2525

Pennington Biomedical Research Center
LOUISIANA STATE UNIVERSITY

July 28, 1992

Gerald S. Berenson, M.D.
Director, National Center for
Cardiovascular Health
Tulane School of Public Health
1430 Tulane Avenue
New Orleans, LA 70112-2699

Dear Dr. Berenson:

Please review the attached correspondence. Colonel Askew requests a final report on the Fort Polk Heart Smart Project. Therefore, I will revise my prior request to you (that asked for a final project report to be submitted as part of the overall grant Final Report).

Colonel Askew states in his letter that the project "is of considerable importance to the Army, (and) we would like to have a report detailing goals, methods, results and recommendations."

I spoke to Cathy Champagne today and she assured me that all of the ETNV data had been forwarded to Theresa Nicklas. Please let me know if there is other information that I can provide that will expedite this report.

Sincerely,

Donna H. Ryan, M.D.
Associate Executive Director

jgw

cc: Colonel Askew

6400 Perkins Road, Baton Rouge, Louisiana 70808-4124 ♦ Phone: (504) 763-2500, Fax: (504) 763-2525

Table 1

FORT POLK HEART STUDY

FORT POLK, LOUISIANA

5TH MECHANIZED DIVISION

15,000 Active Duty Personnel

10,000 Personnel With Depdenents

6,000 Child Dependents

Table 2

FORT POLK HEART STUDY

Project 1 - Baseline Assessment of Dietary Intake and Physical Activity in Military Dependents

Sample - 200 Wives of Military Personnel With At Least 1 Child

Goals - Characterize Eating, Food Purchasing, and Physical Activity Patterns

Measures

1. 24-Hour Dietary Recall
2. Food Purchasing Questionnaire
3. Pantry Survey
4. Physical Activity Recall
5. Health Habits Questionnaire
6. CVD Risk Factor Screening

Table 3

FORT POLK HEART STUDY

Project 2 - Cardiovascular Risk Assessment of Families at Fort Polk

Sample - 100+ Complete Families of Fort Polk Personnel

Goals - Establish Norms for CVD Risk Factors

Measures

1. Blood Pressure
2. Blood Lipids
3. Anthropometry
4. Medical History Questionnaire
5. Health Habits Questionnaire

Table 4

FORT POLK HEART STUDY

Project 3 - Family Health Promotion

Sample - 60 Complete Families of Fort Polk Personnel

Goals - Develop a Heart Health Education Model For Military Families

Measures and Procedures

1. CVD Risk Factor Screening
2. Eating, Physical Activity, and Behavior Modification Counseling
3. Health Habits Questionnaire

FORT POLK HEART SMART PROGRAM - ATTACHMENT A-1

FINAL REPORT - AUGUST, 1991

FORT POLK HEART SMART PROGRAM - ATTACHMENT A-2

FINAL REPORT - AUGUST, 1991

TABLE A-1

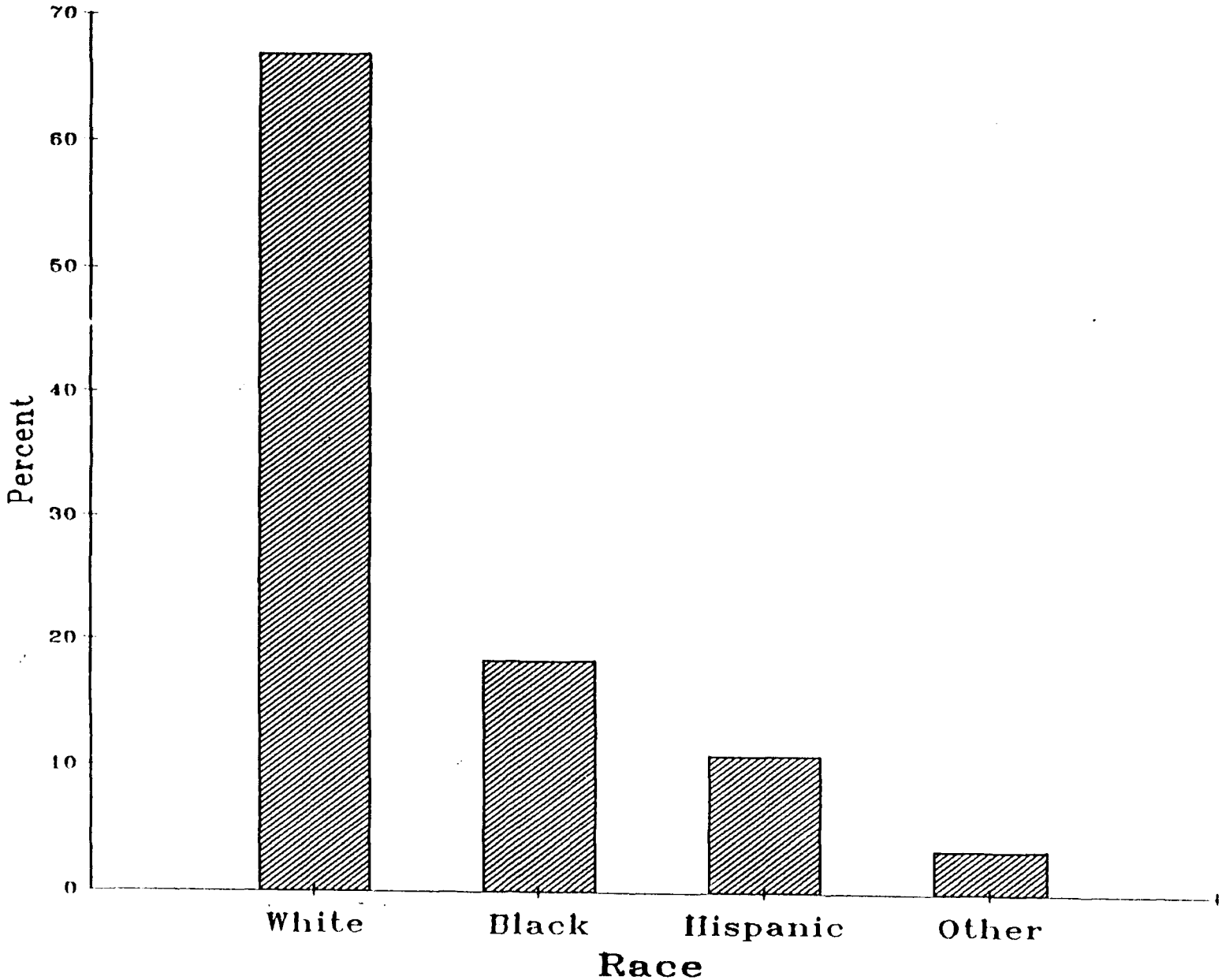
NUMBER OF SUBJECTS EXAMINED
FORT POLK HEART SMART PROGRAM

AGE

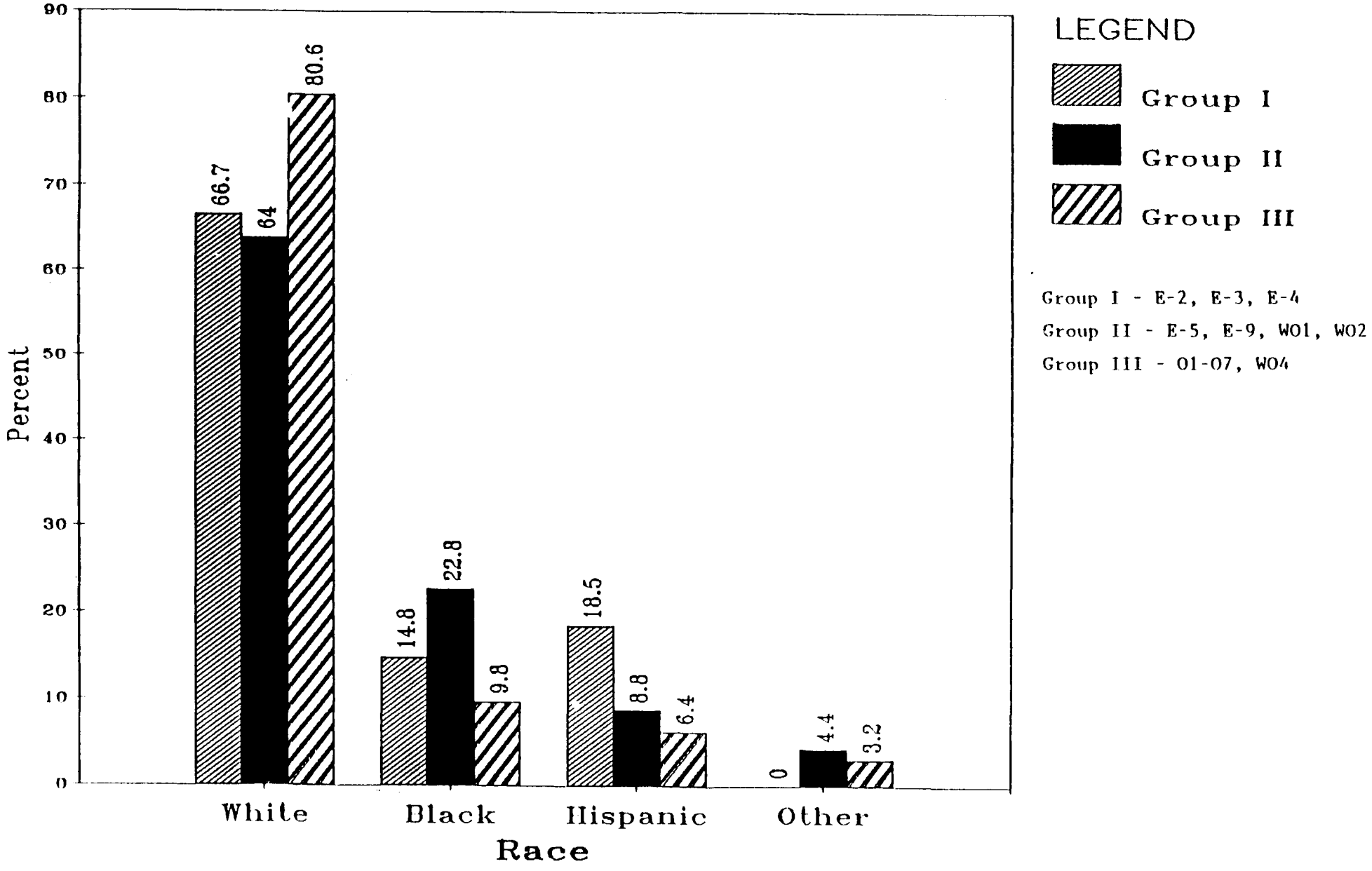
	0-9	10-19	20-29	30-39	40+	TOTAL
White Males	29	30	53	56	9	177
White Females	20	28	128	79	20	275
Black Males	9	10	25	21	5	70
Black Females	7	14	39	32	5	97
Hispanic Males	2	0	6	6	0	14
Hispanic Females	2	1	17	12	0	32
Other Males	3	4	2	0	1	10
Other Females	4	6	8	9	1	28
Total Males	43	44	86	83	15	271
Total Females	33	49	191	132	26	432
TOTAL	76	93	277	215	41	703

Race Distribution Among Military Wives
Fort Polk Heart Smart Program, 1989

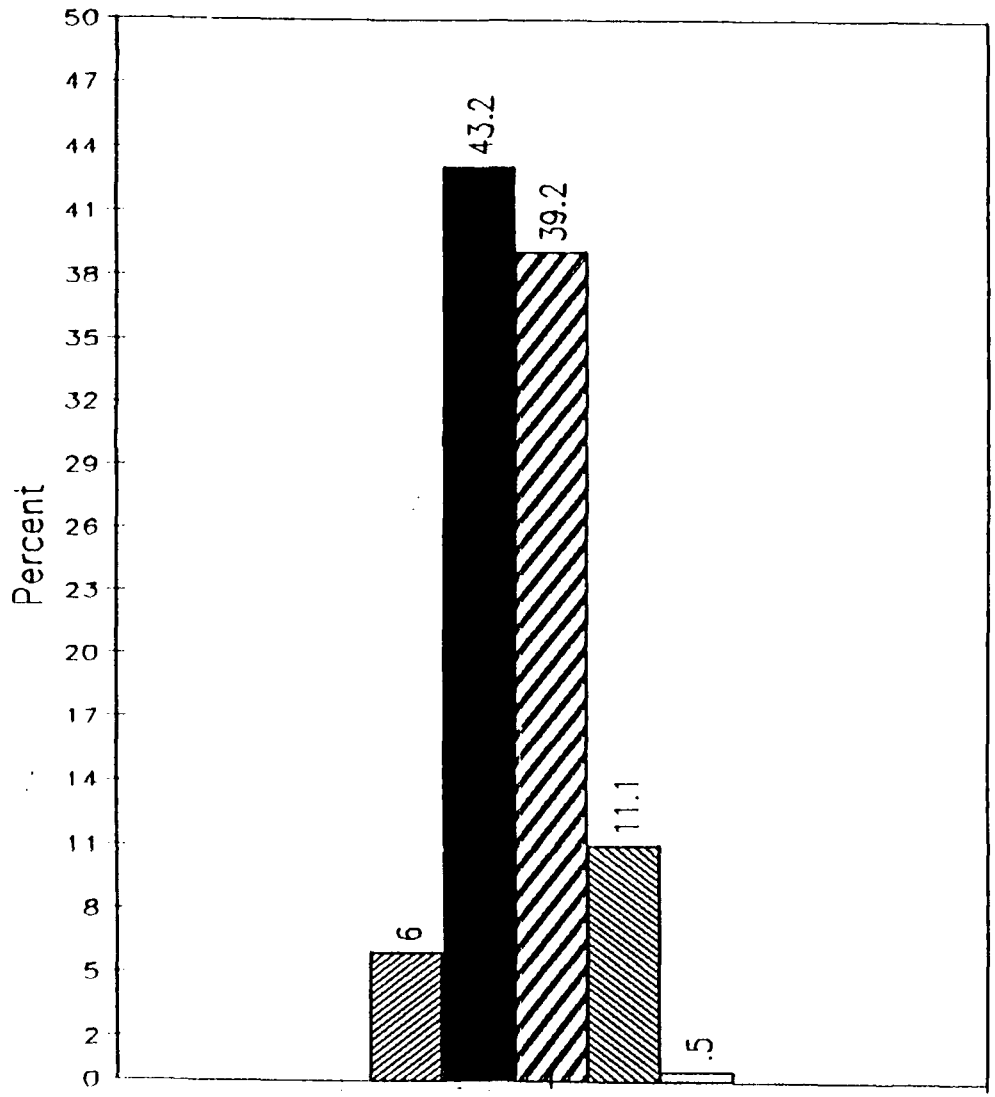
N = 200



Rank and Race of Husbands
Fort Polk Heart Smart Program, 1989



Education of Fort Polk Spouses
Fort Polk Heart Smart Program, 1989

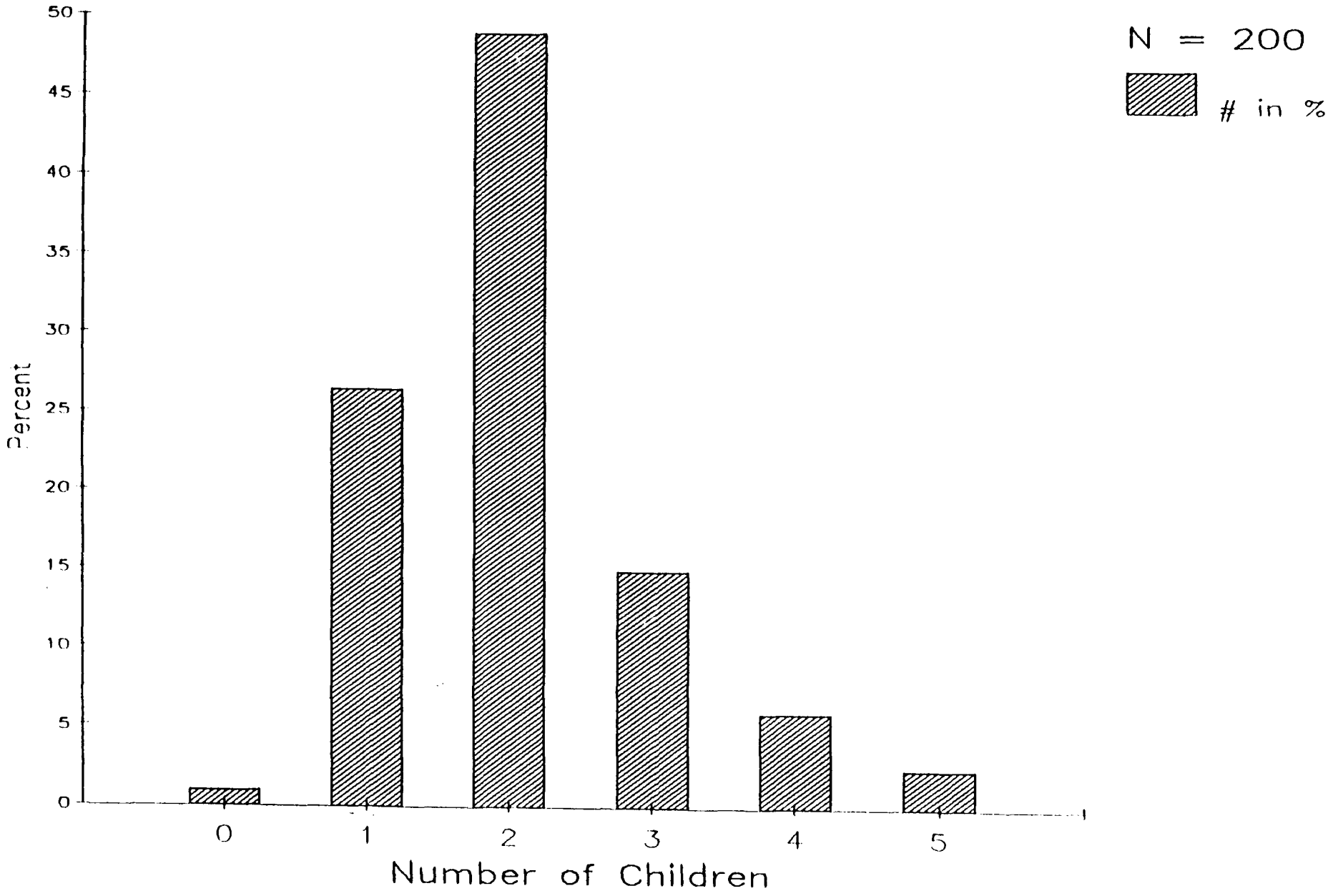


LEGEND

- Non High School Graduate
- High School Graduate
- Some College or Trade School
- College Degree
- Advanced Degree

N=199

Children of Military Wives
Fort Polk Heart Smart Program, 1989



Distribution of Husband's Rank for WIC and Food Stamp Users
Fort Polk Heart Smart Program, 1989

WIC

Rank	N	%
E-1	0	0
E-2	0	0
E-3	3	1.51
E-4	10	5.03
E-5	5	2.51
E-6	3	1.51
Total	21	10.55

Food Stamps

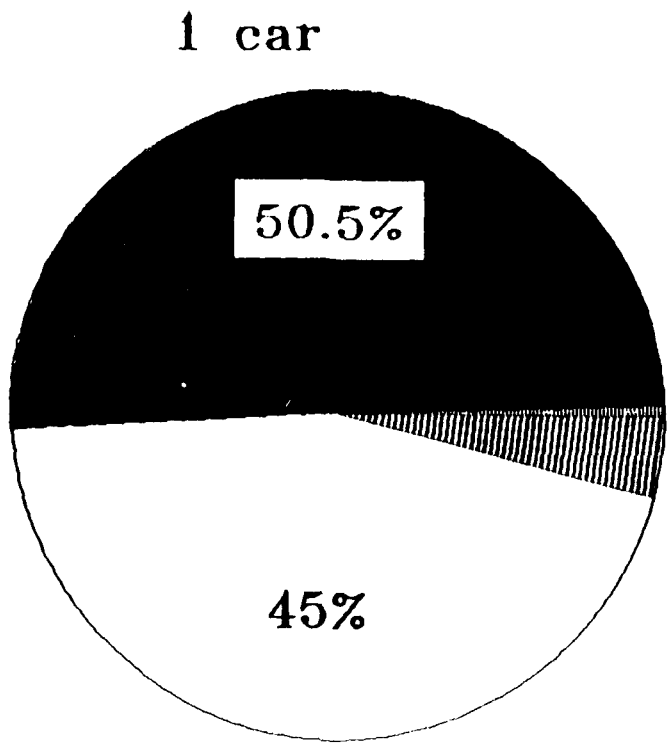
Rank	N	%
E-1	0	0
E-2	0	0
E-3	1	0.5
E-4	2	1.01
E-5	0	0
E-6	0	0
Total	3	1.51

Frequency Distribution for WIC and Food Stamps
Fort Polk Heart Smart Program, 1989

	WIC	
	N	%
Yes	21	10.5
No	179	89.5

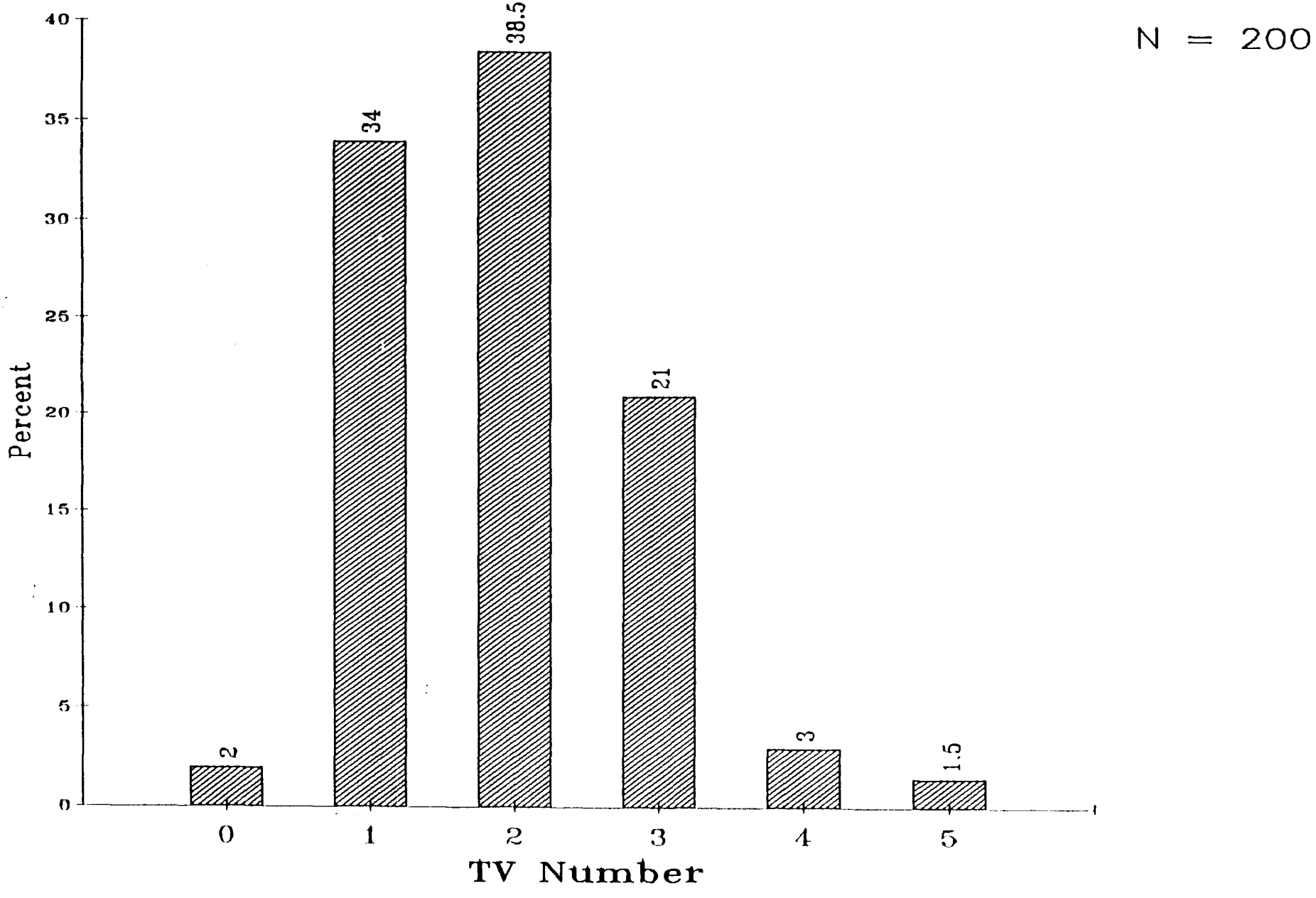
	Food Stamps	
	N	%
Yes	3	1.5
No	197	98.5

Number of Cars Per Family
Fort Polk Heart Smart Program, 1989

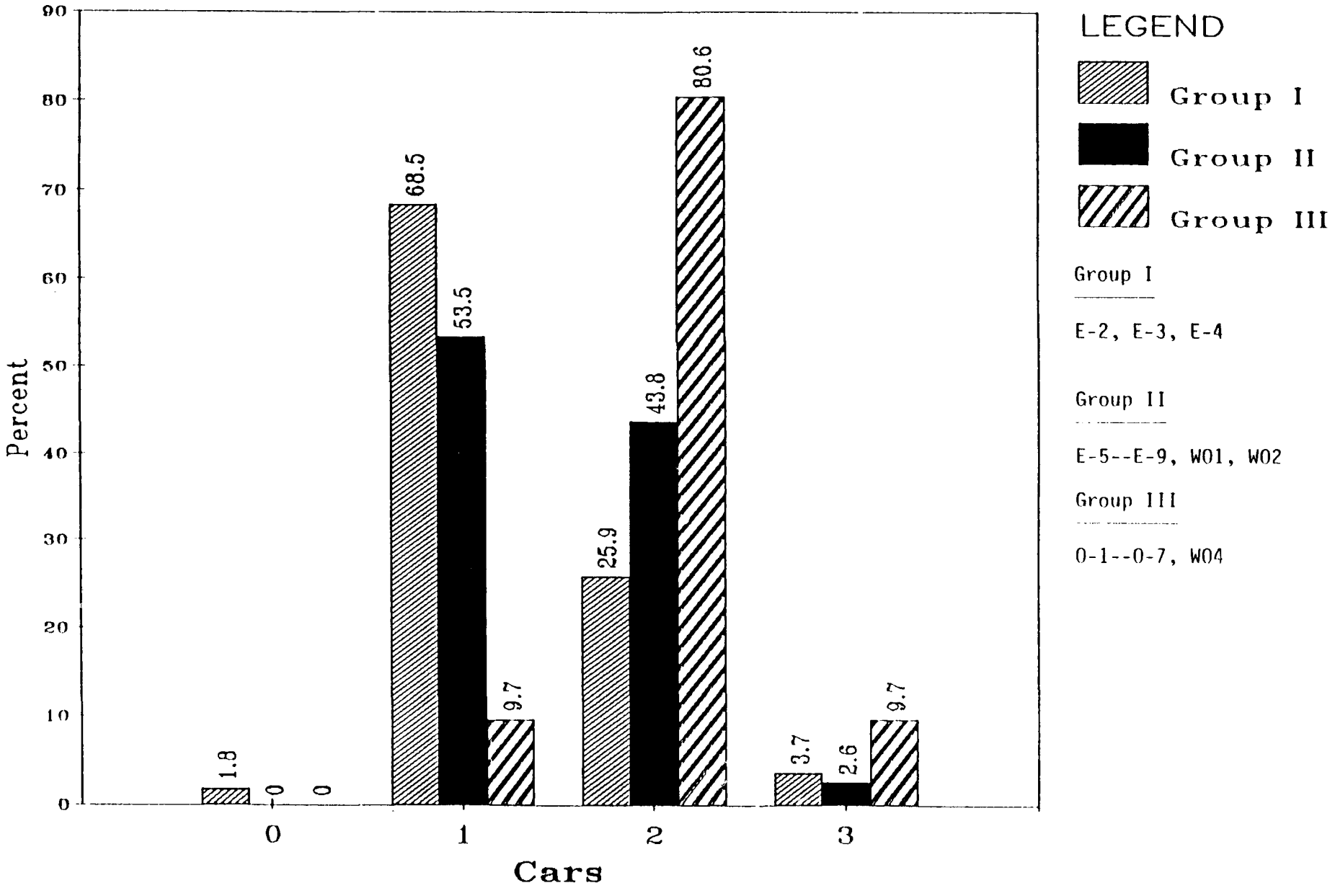


N = 200

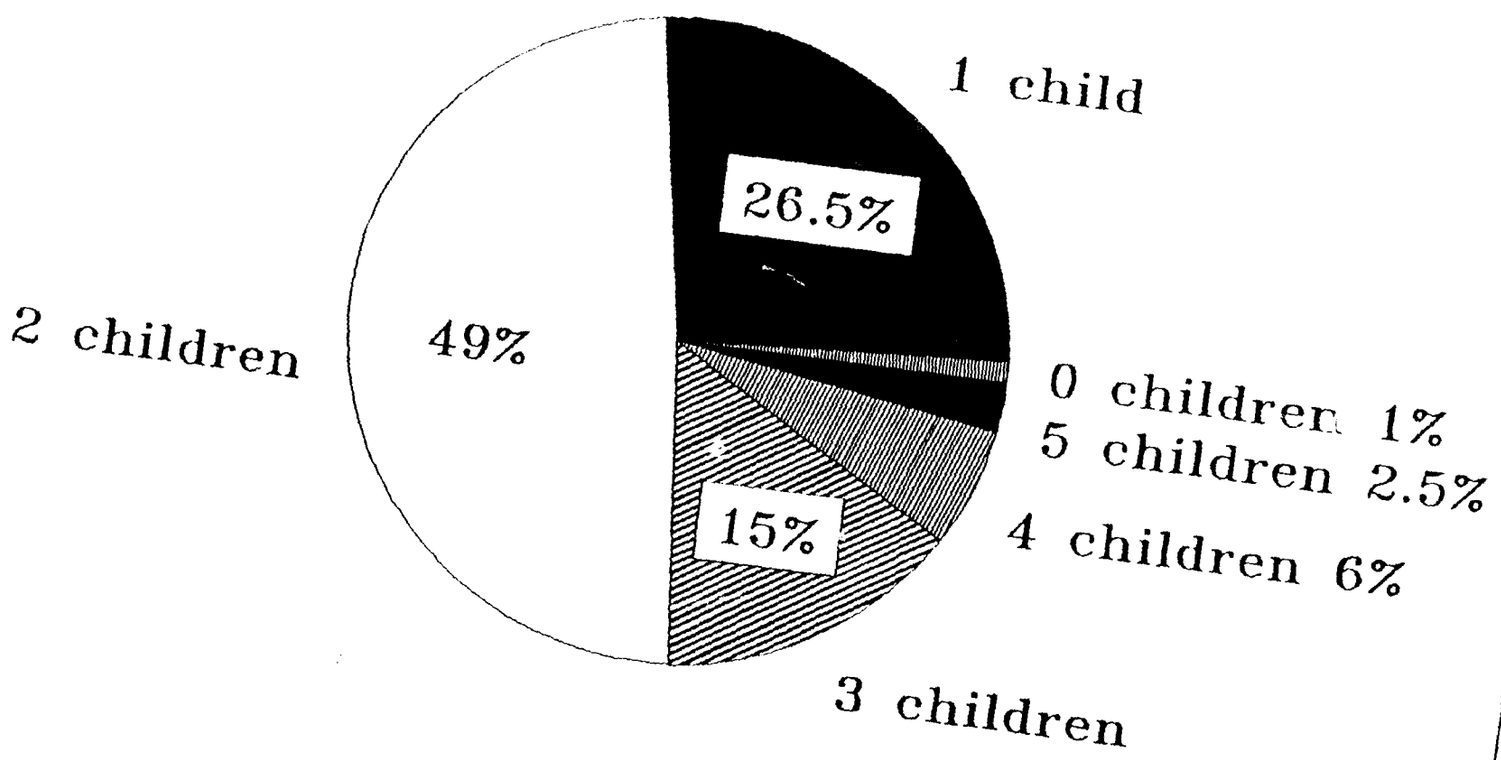
Number of Televisions Per Family
Fort Polk Heart Smart Program, 1989



Ranks of Husbands and Number of Cars Owned
Fort Polk Heart Smart Program, 1989

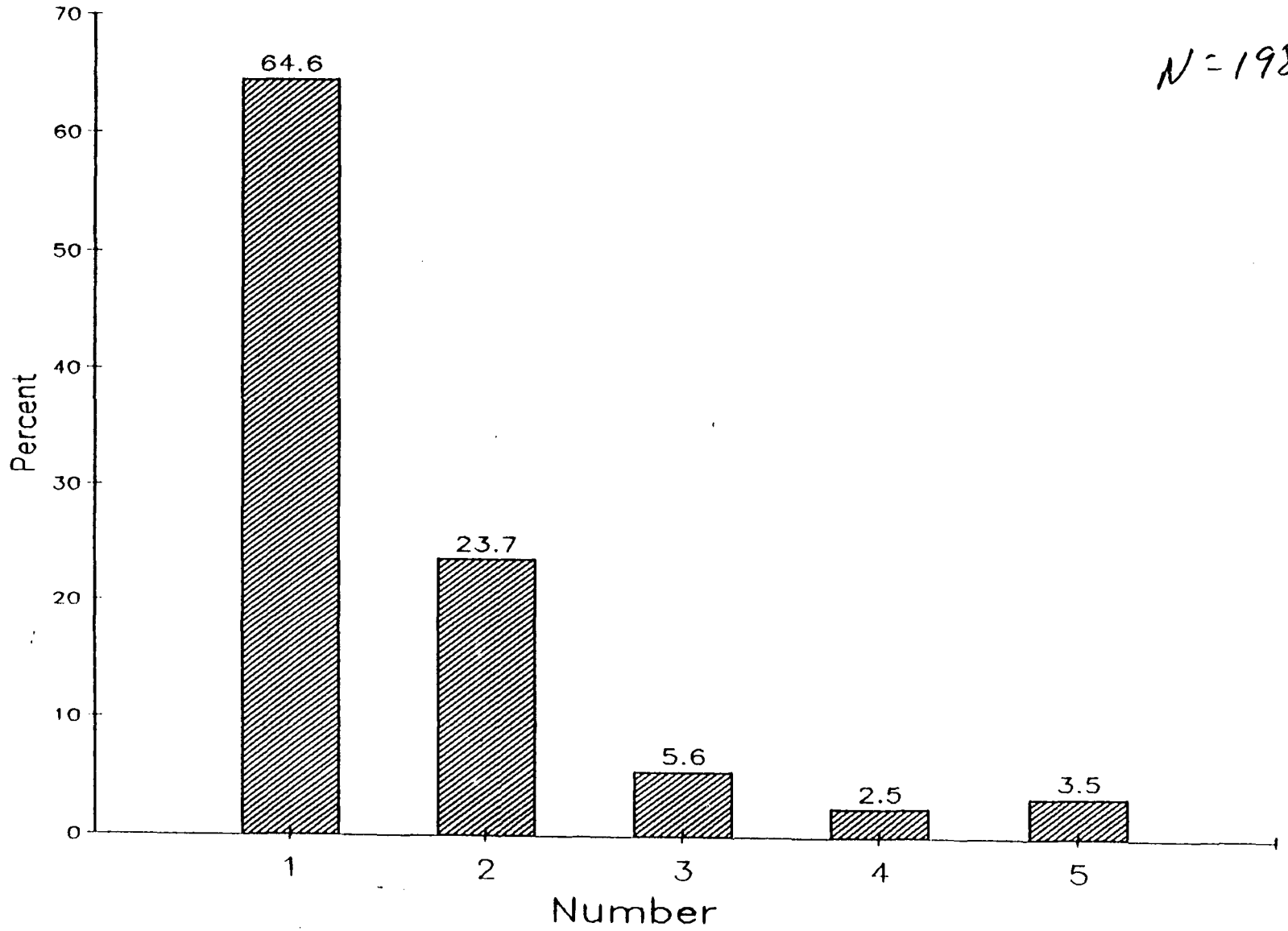


Number of Children Per Family
Fort Polk Heart Smart Program, 1989

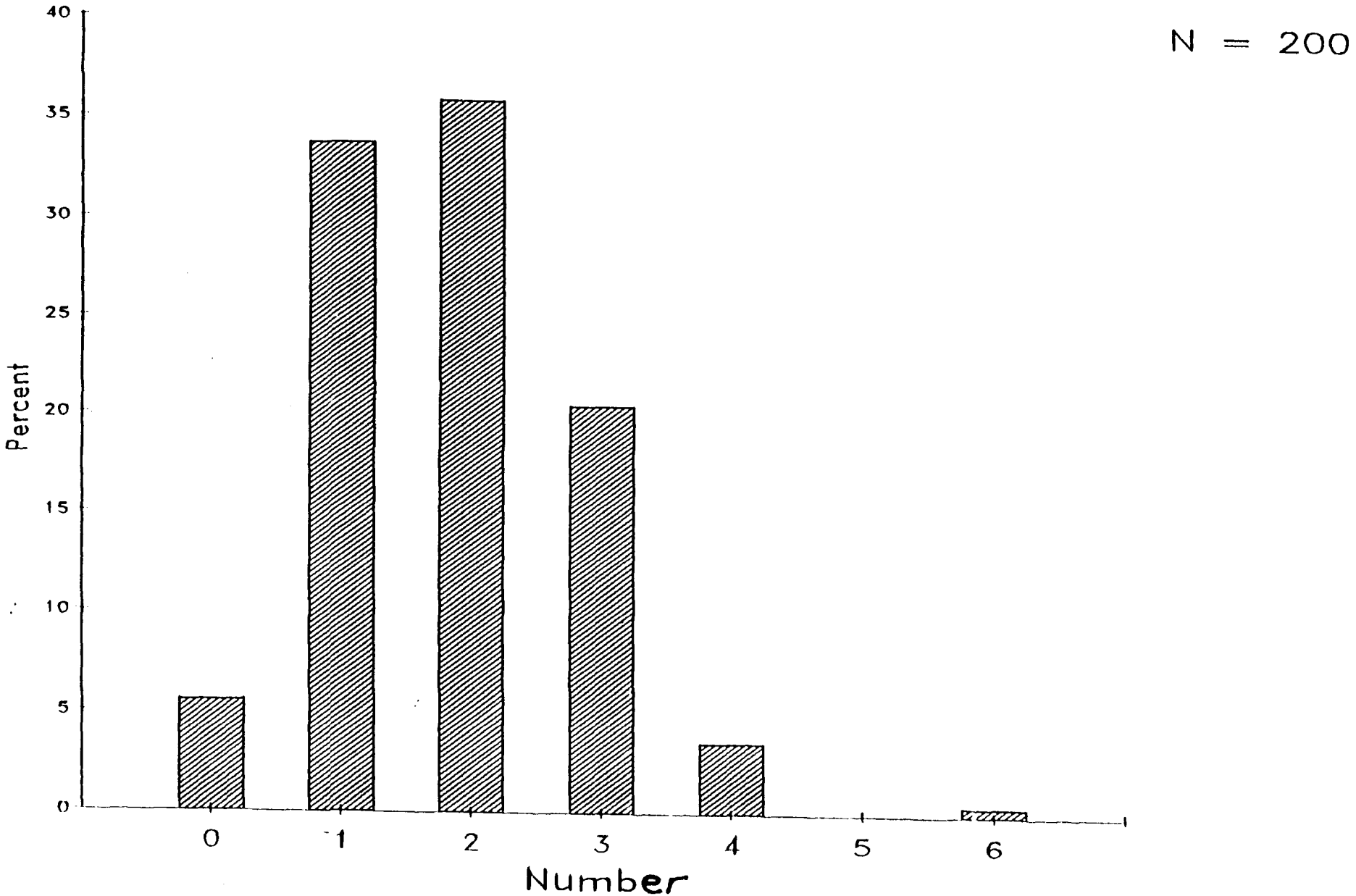


N = 200

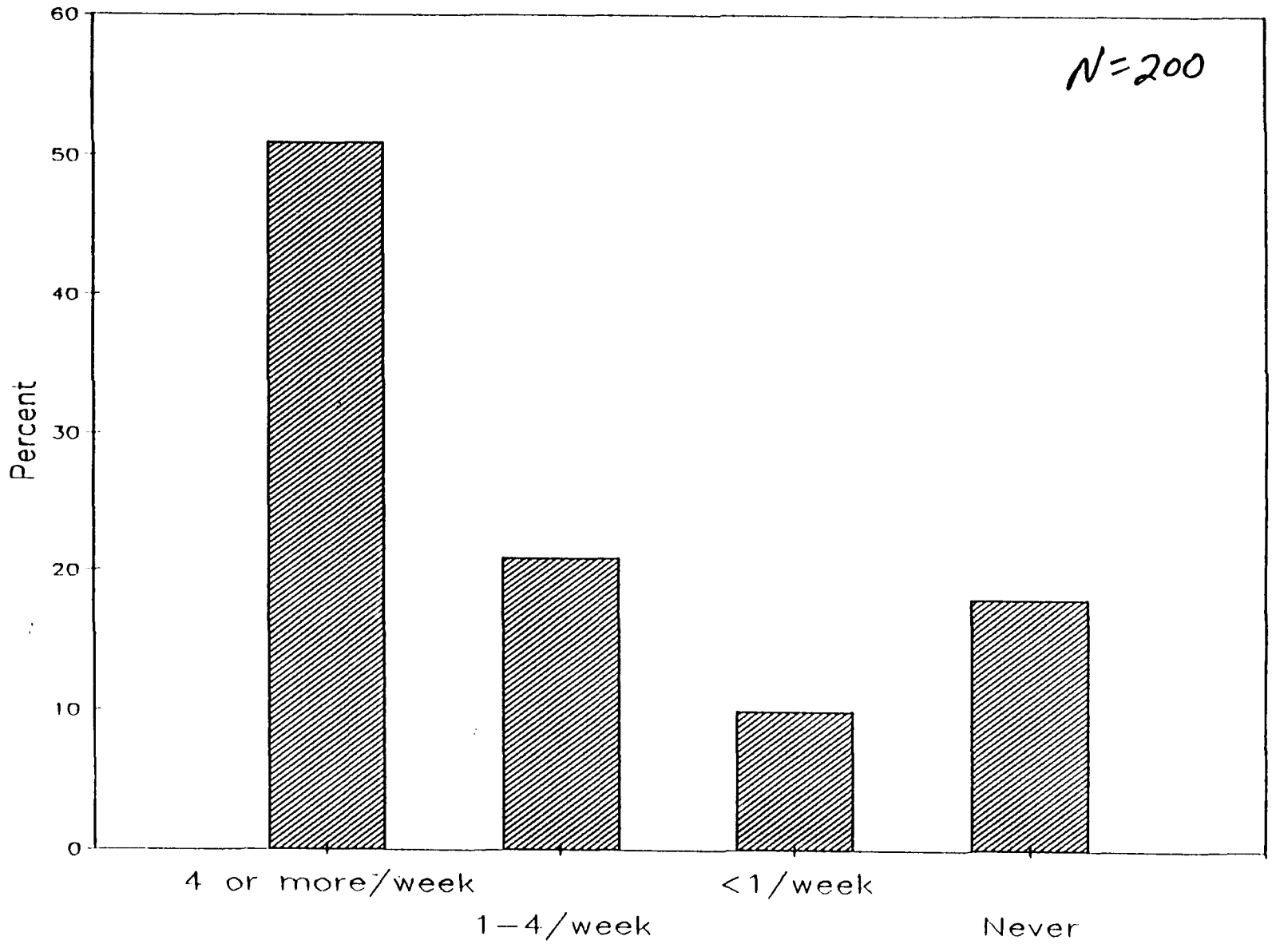
Family Meals Eaten Together Per Day
Fort Polk Heart Smart Project, 1989



Number of Snacks Eaten Per Day
Fort Polk Heart Smart Program, 1989



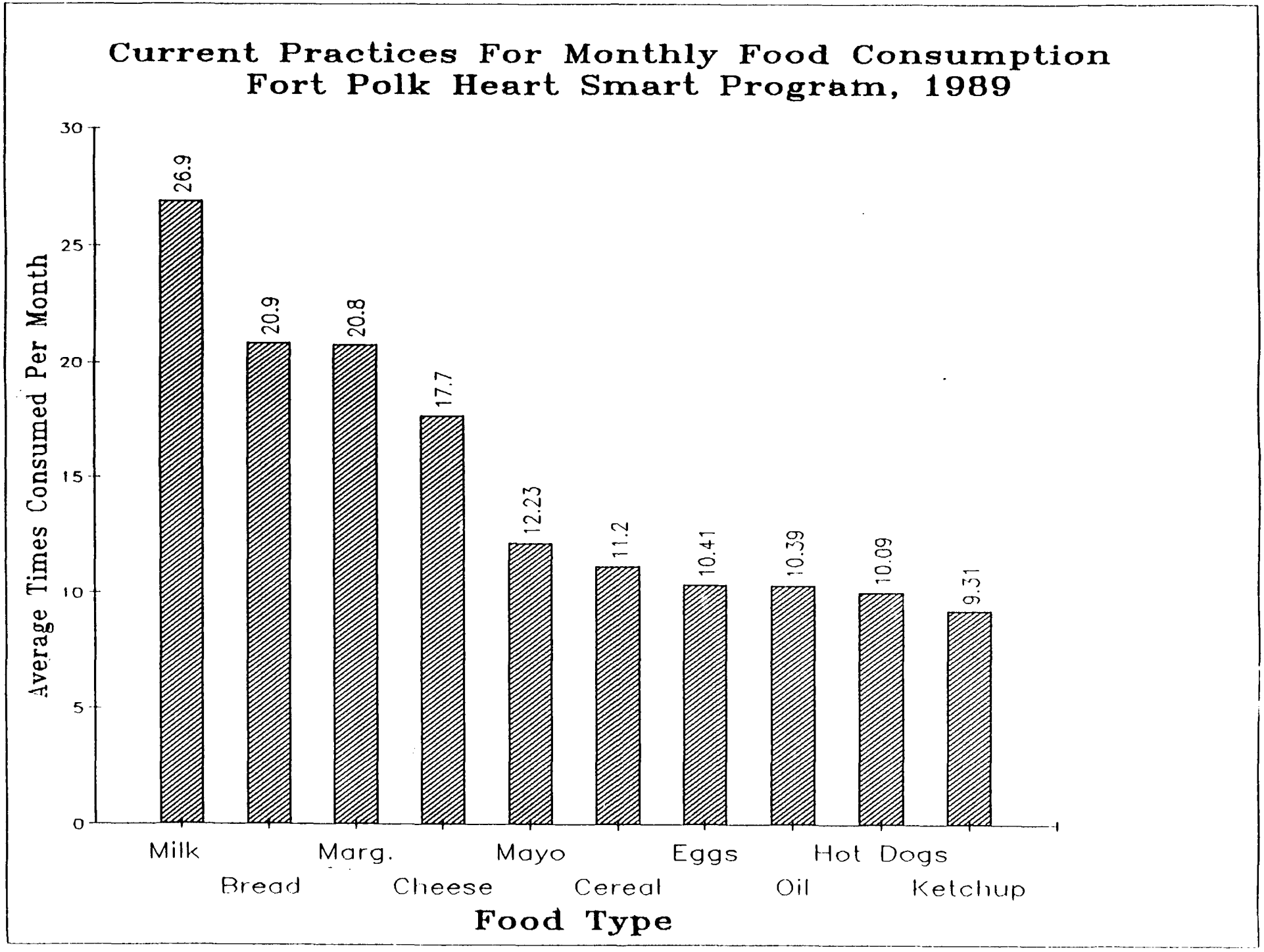
Meals Eaten While Watching TV
Fort Polk Heart Smart Program, 1989



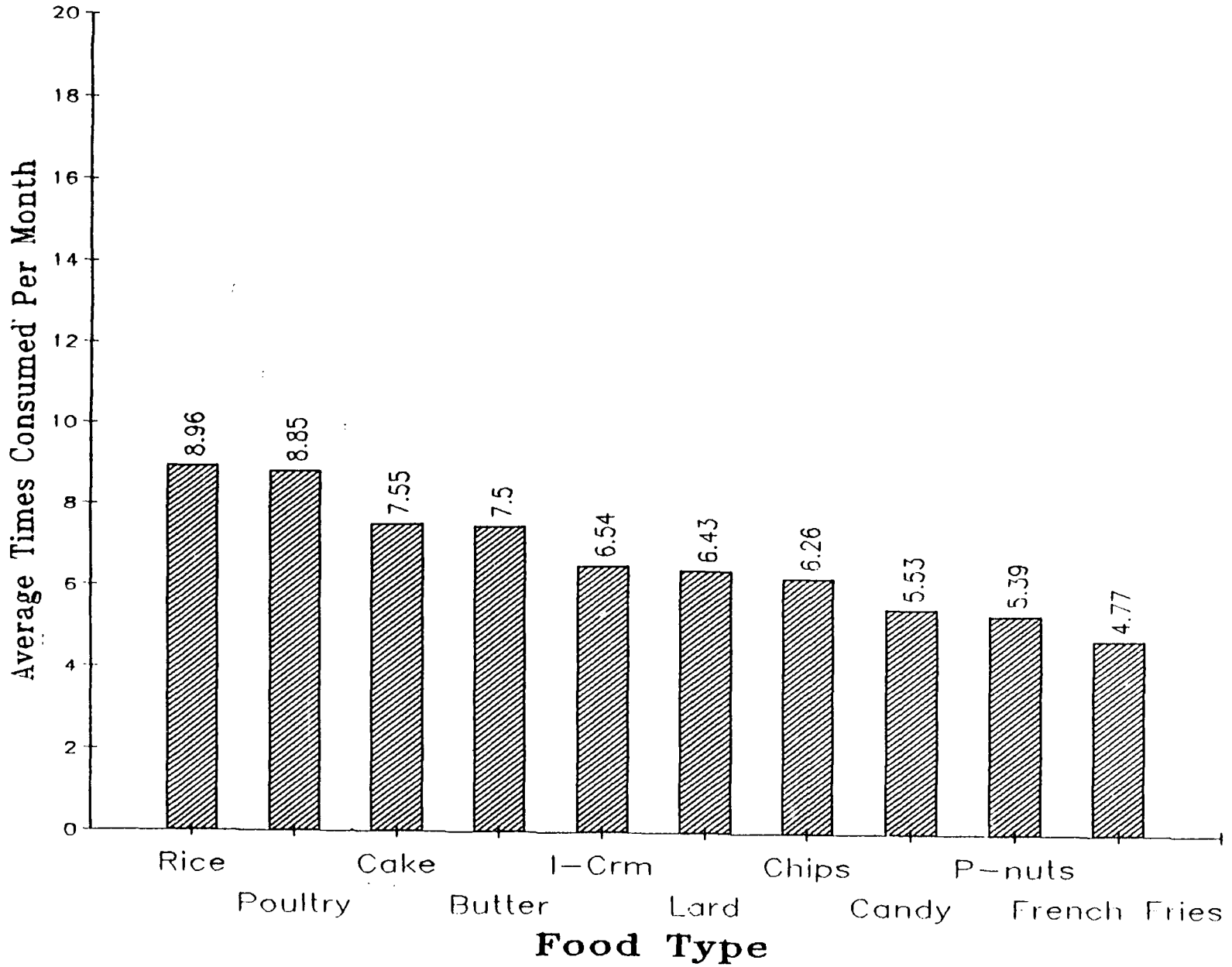
FORT POLK HEART SMART PROGRAM

Frequency Scoring

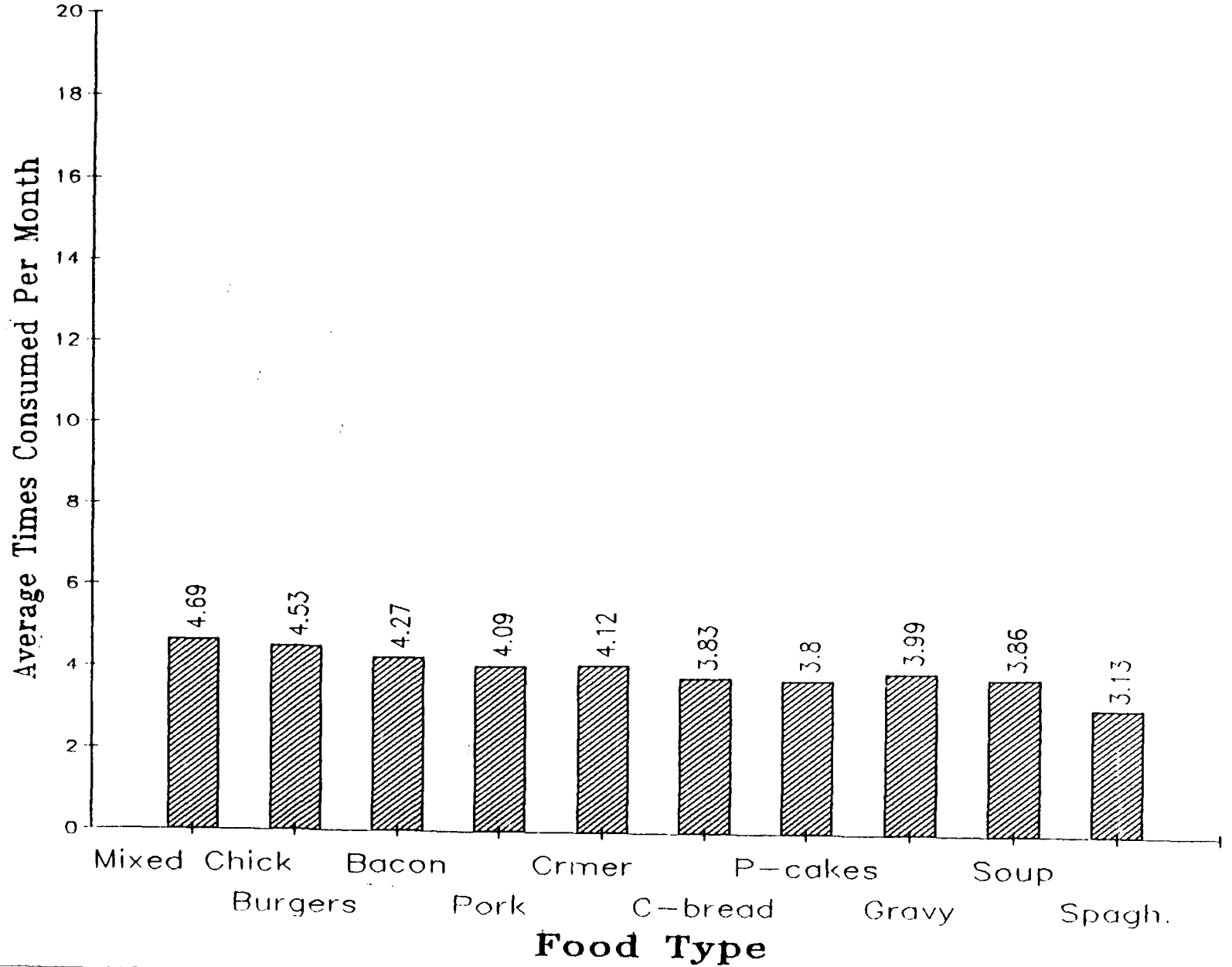
Frequency	Score
Never	0
Less than 1 time per month	.5
About 1 time per month	1
About 2-3 times per month	2.5
About 1 time per week	4
Several times per week	14
One time a day	30
More than 1 time per day	45


Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
30
25
20
15
10
5
0
26.9
20.9
20.8
17.7
12.23
11.2
10.41
10.39
10.09
9.31
Milk
Bread
Marg.
Cheese
Mayo
Cereal
Eggs
Oil
Hot Dogs
Ketchup
Food Type

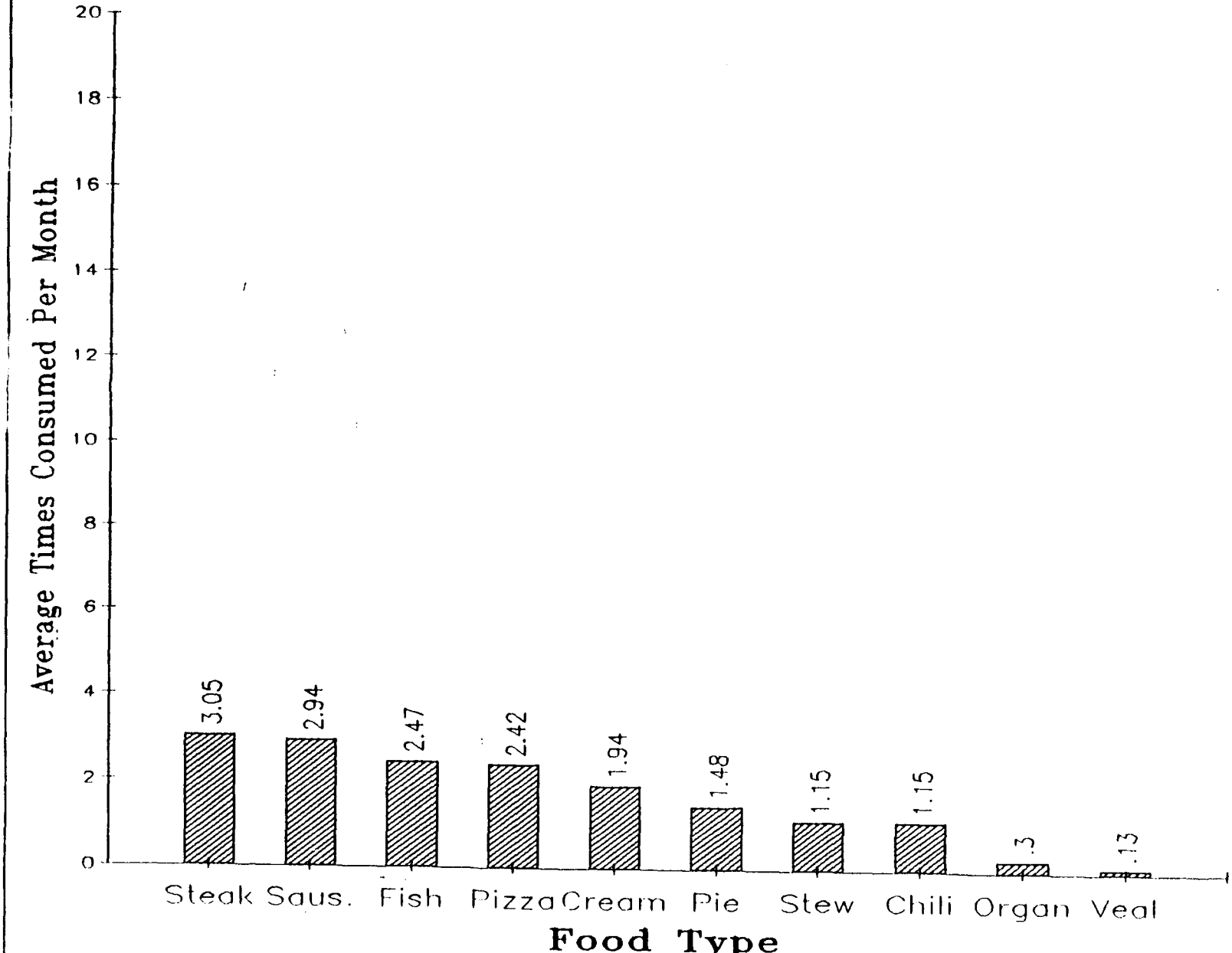
Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989



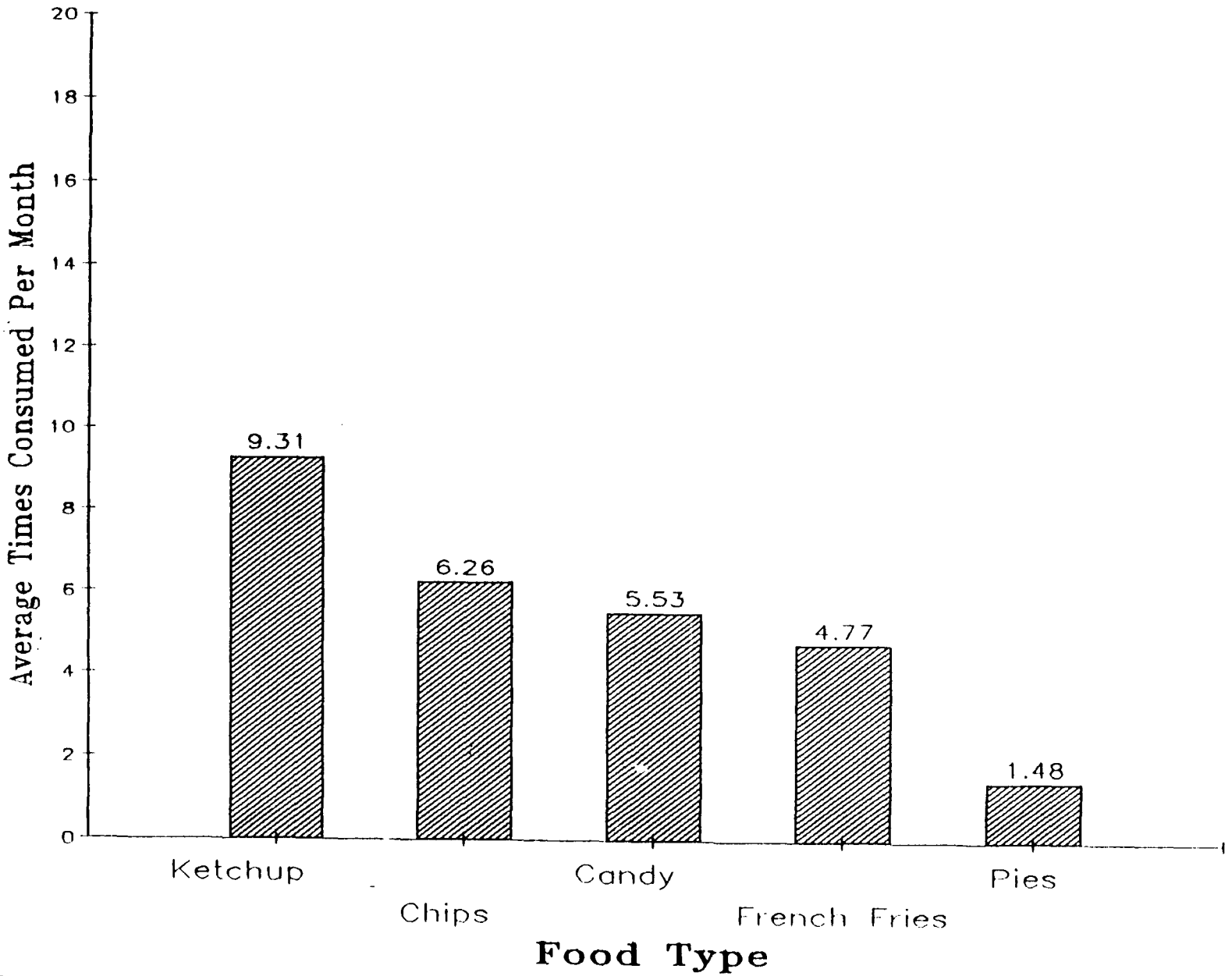
Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989



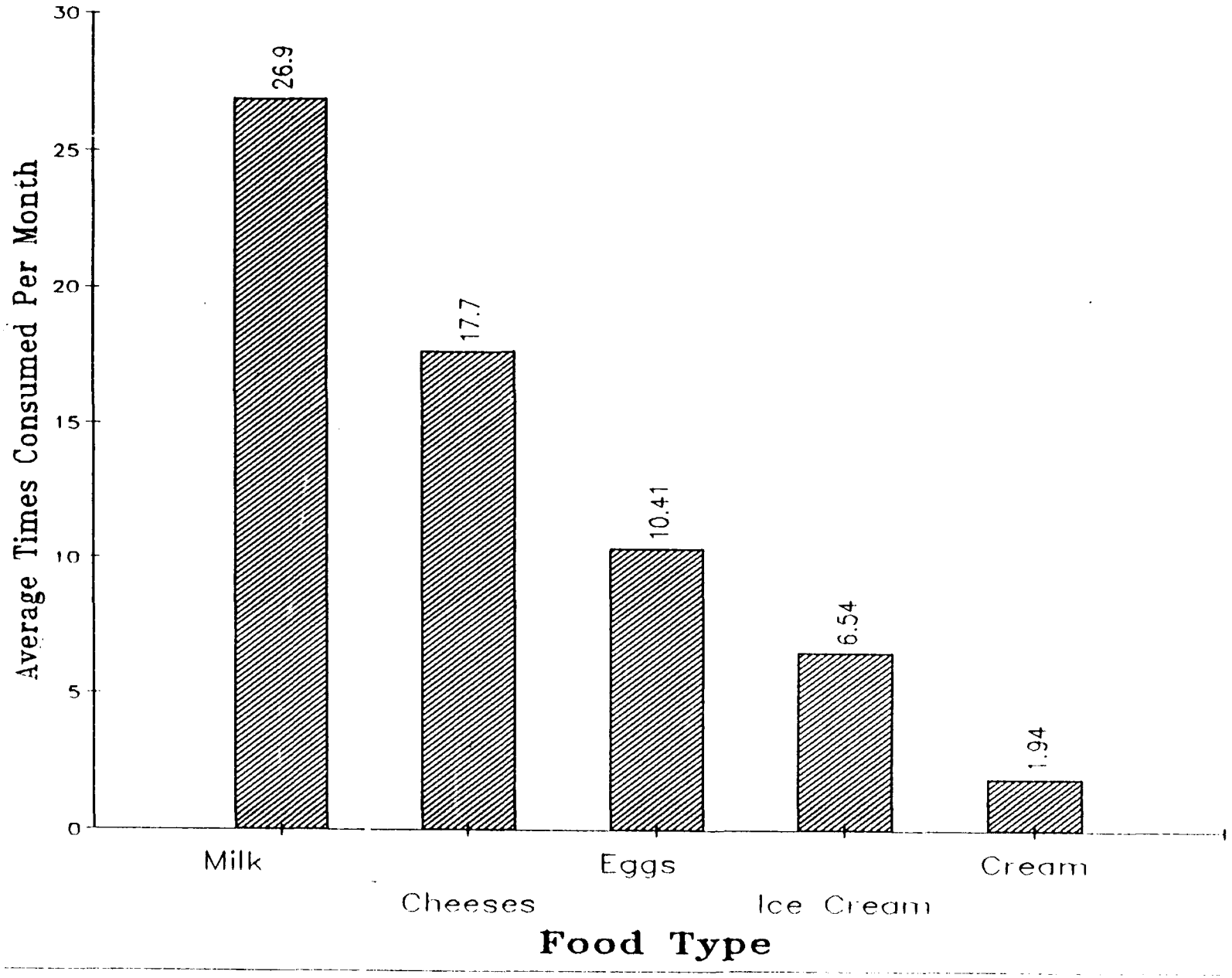
Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989



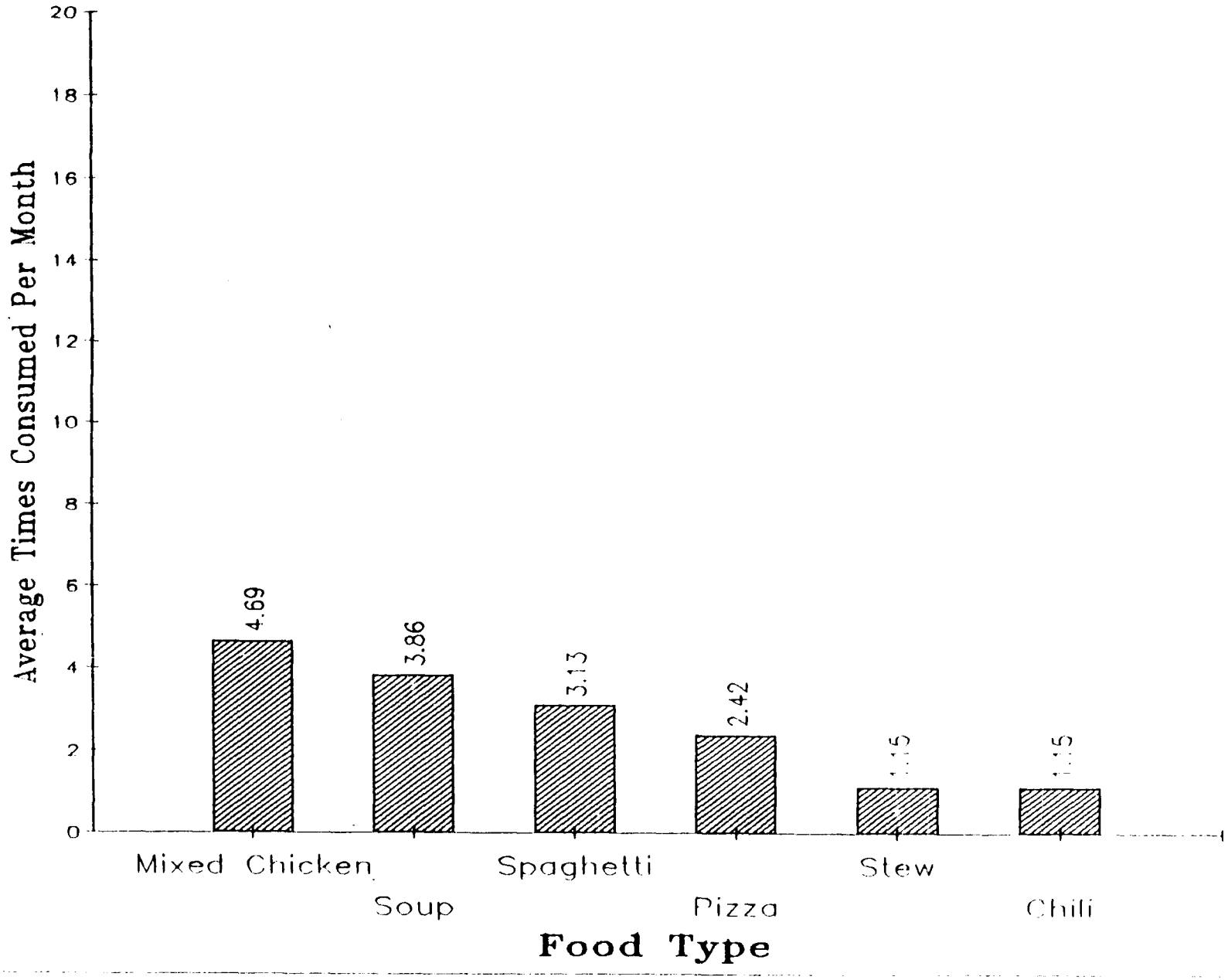
Current Practices For Snacks/Desserts/Misc Consumption
Fort Polk Heart Smart Program, 1989



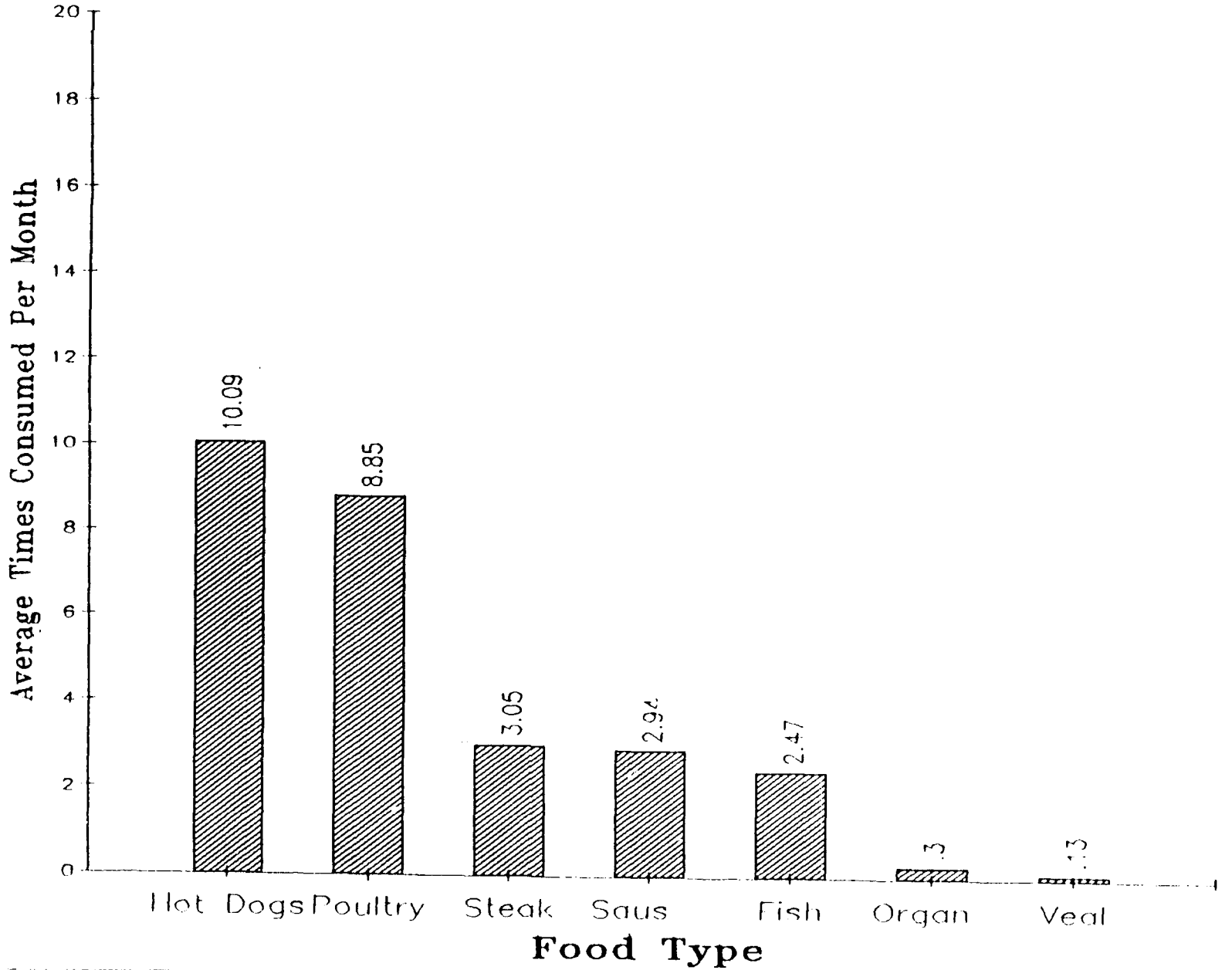
Current Practices For Consumption of Dairy Products
Fort Polk Heart Smart Program, 1989



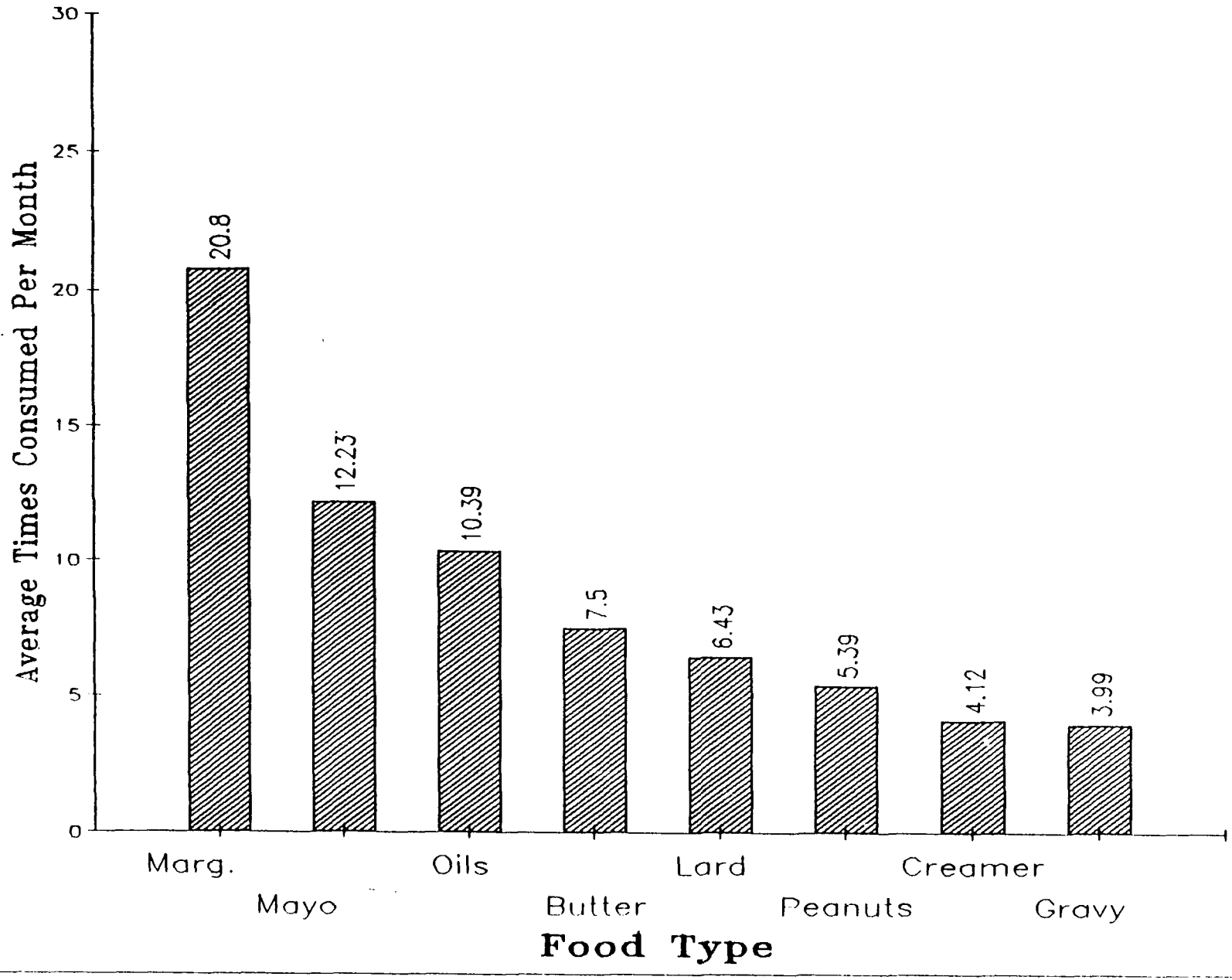
Current Practices For Consumption of Mixed Meat Dishes Fort Polk Heart Smart Program, 1989

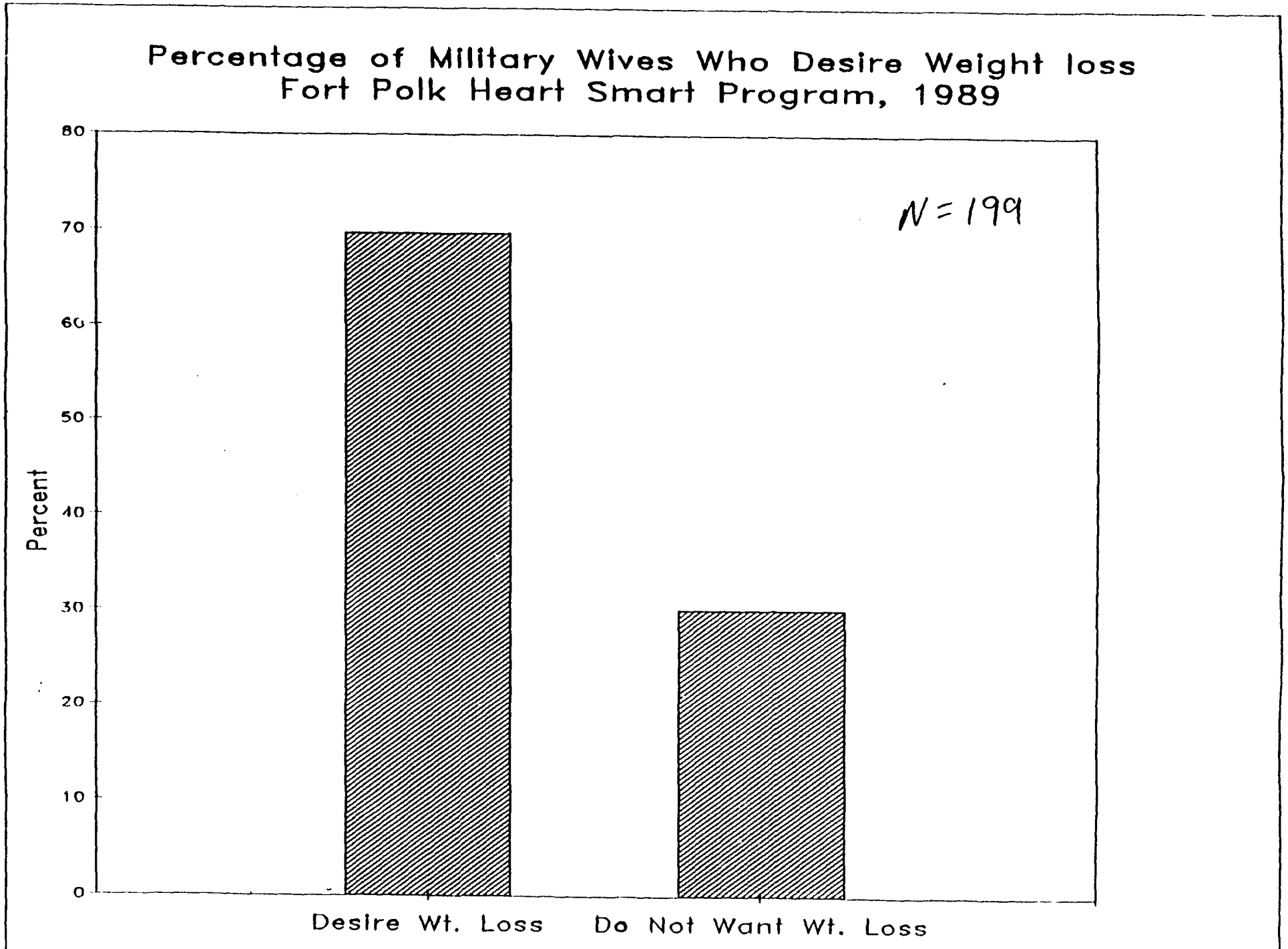


Current Practices For Monthly Meat Consumption
Fort Polk Heart Smart Program, 1989

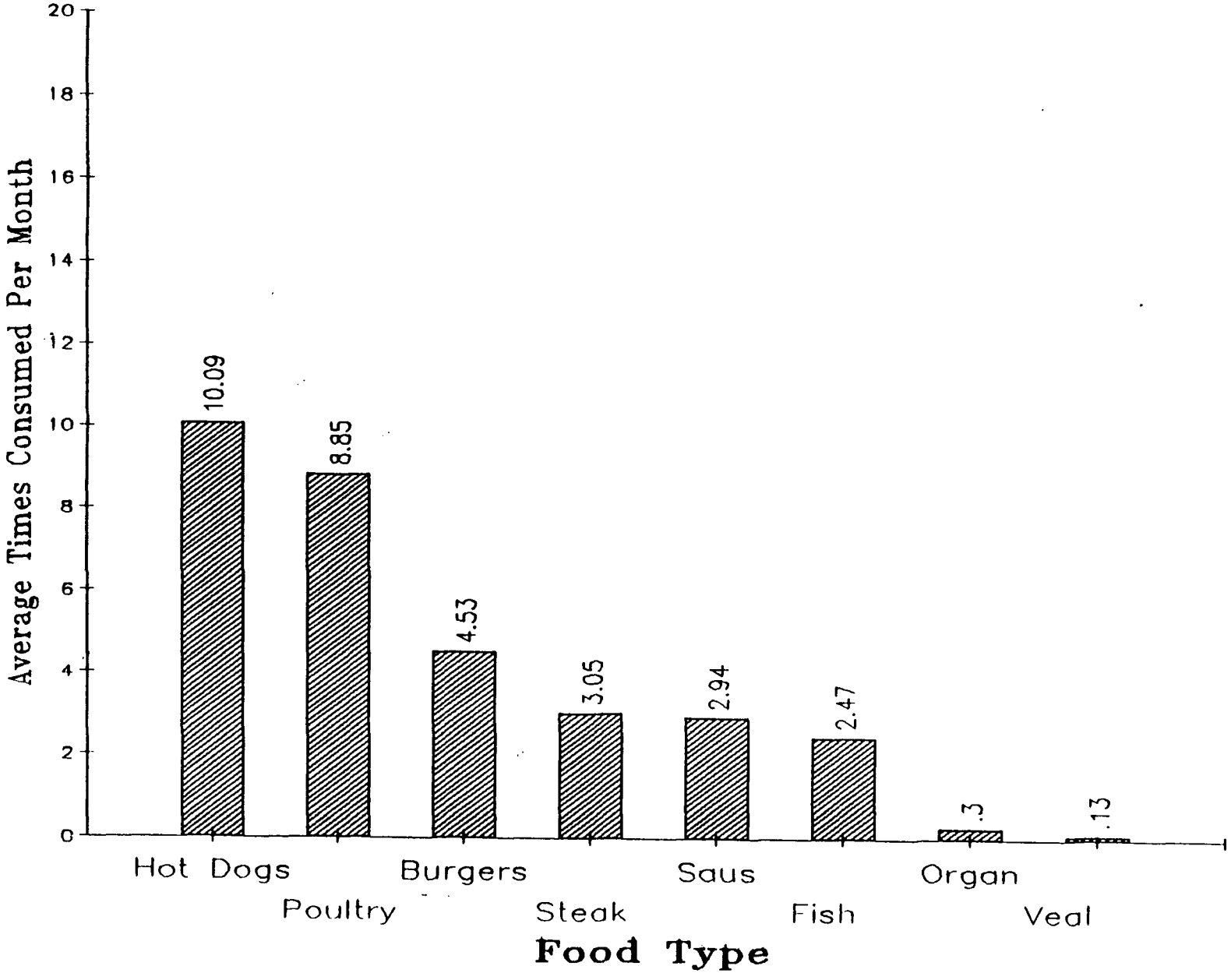


Current Practices For Fats and Oils Consumption
Fort Polk Heart Smart Program, 1989

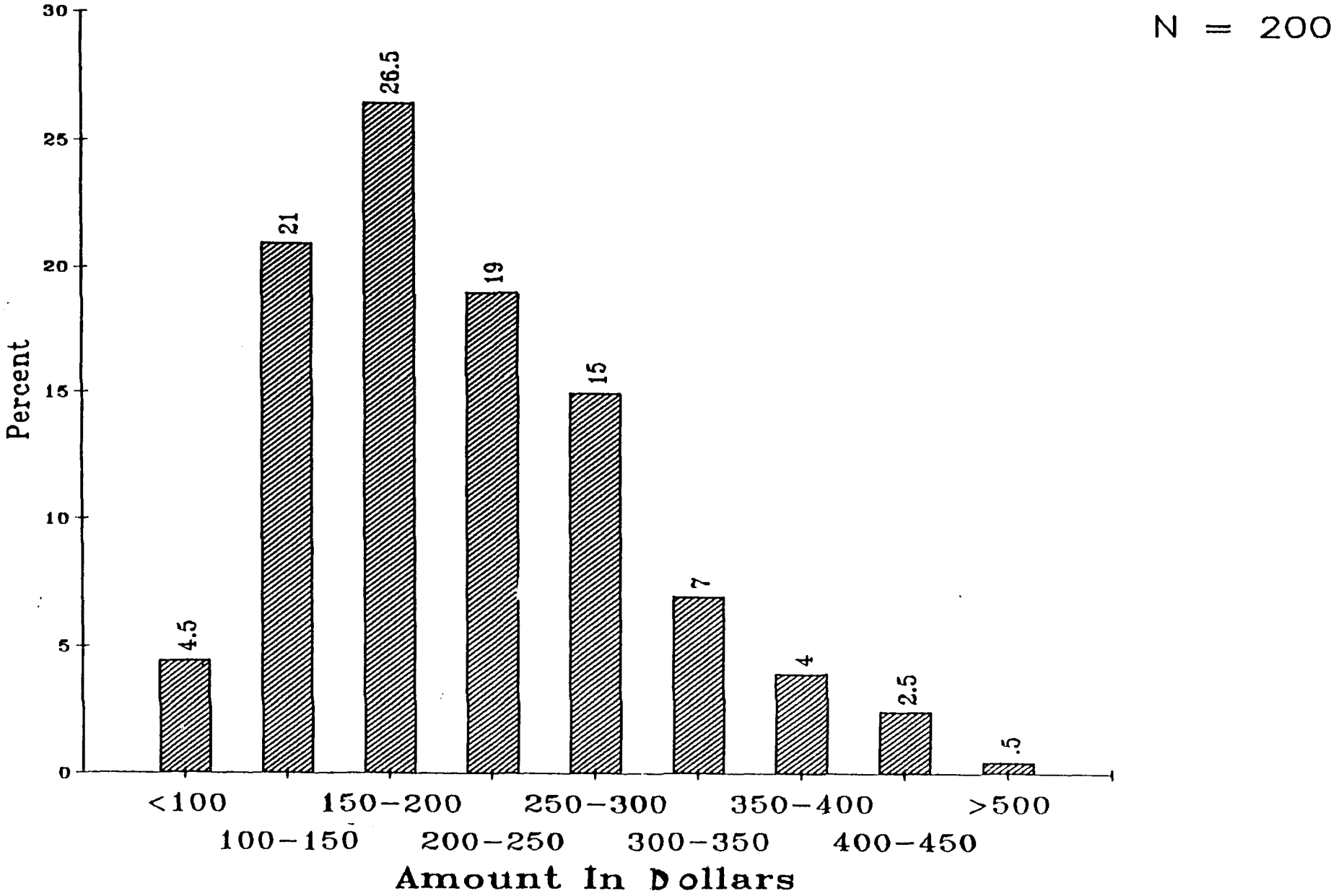



Percentage of Military Wives Who Desire Weight loss
Fort Polk Heart Smart Program, 1989
N = 199
Percent
80
70
60
50
40
30
20
10
0
Desire Wt. Loss
Do Not Want Wt. Loss

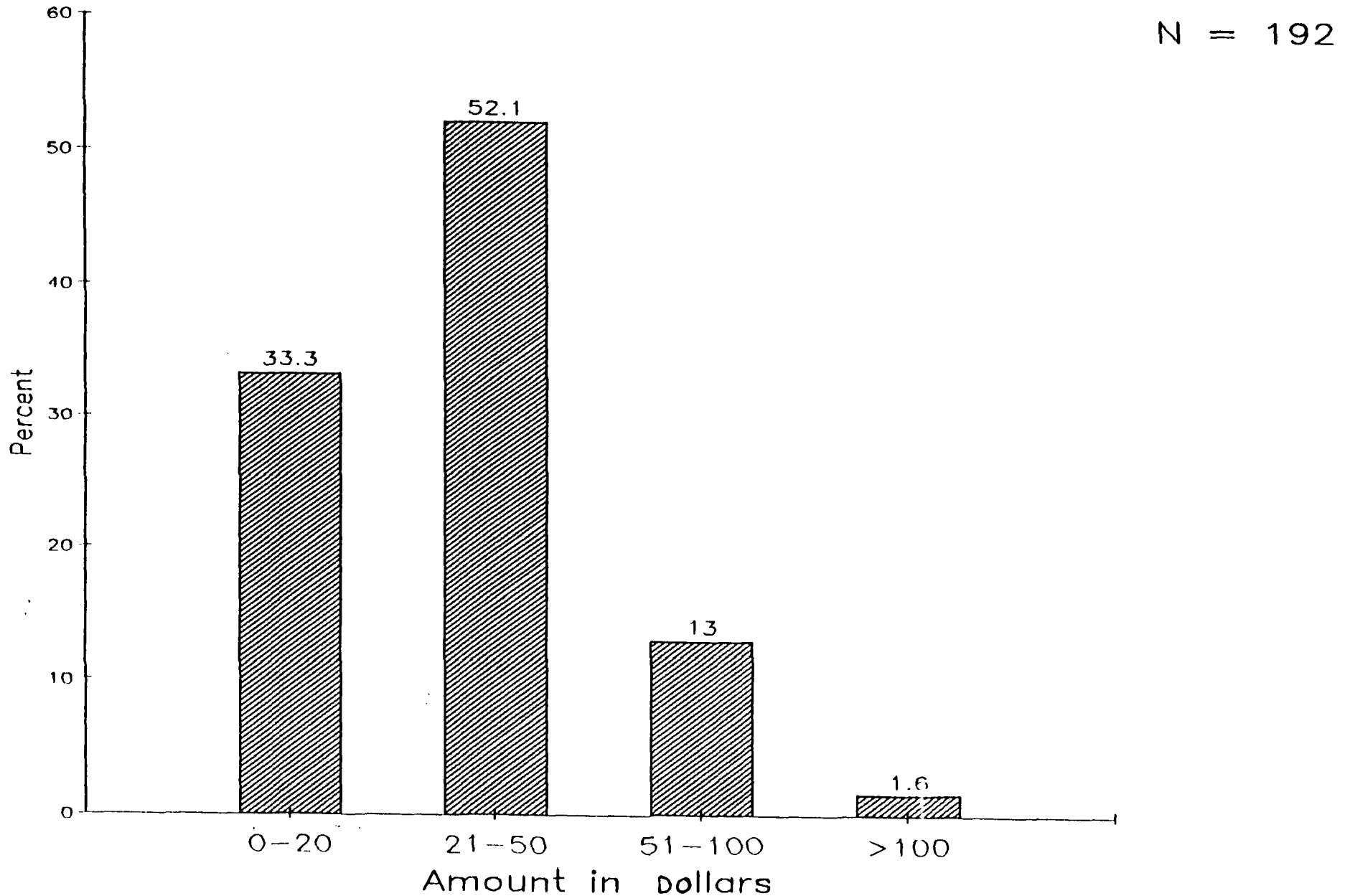
Current Practices For Monthly Meat Consumption
Fort Polk Heart Smart Program, 1989



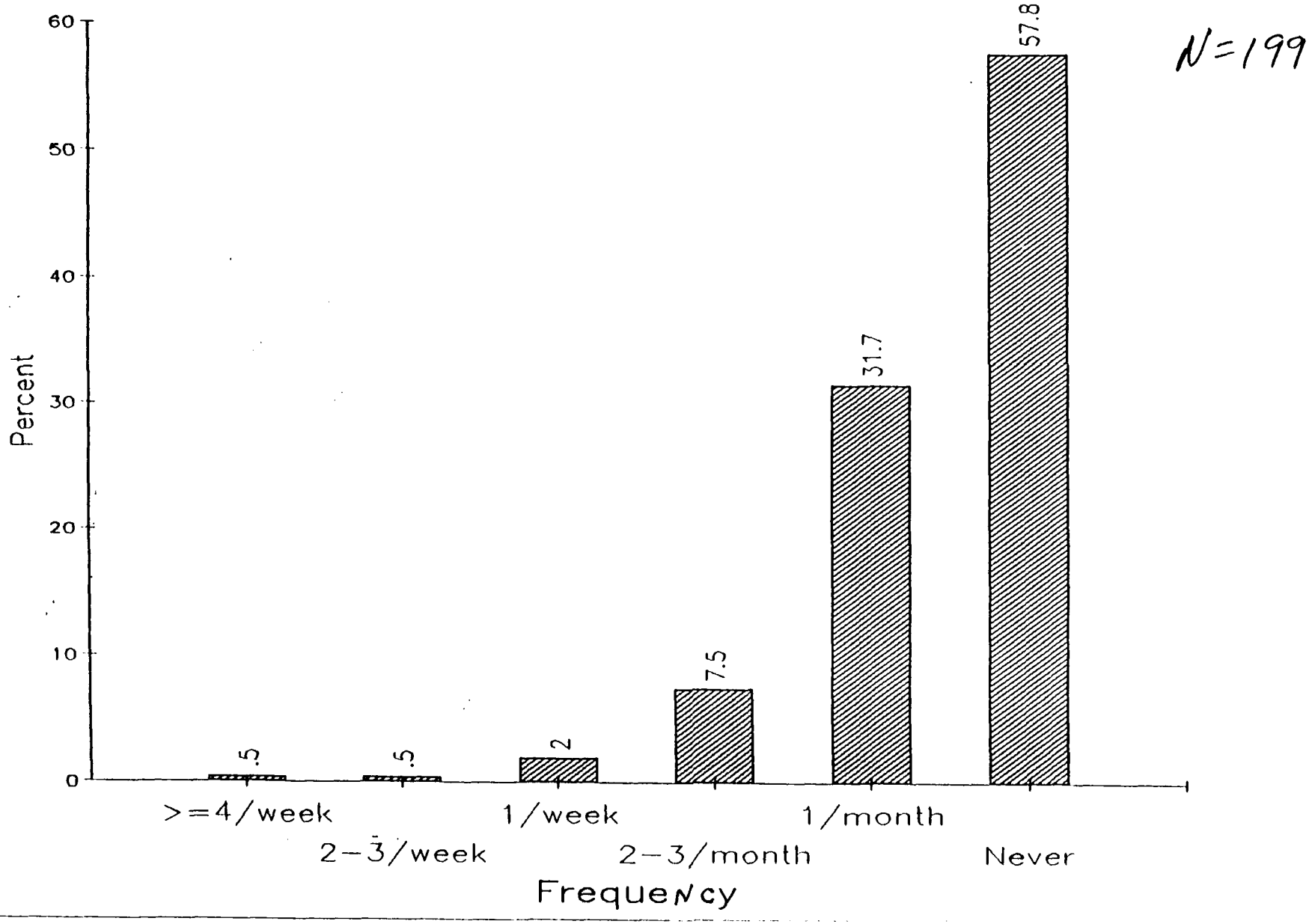
Grocery Expenditures of Military Wives
Fort Polk Heart Smart Program, Project 1, 1989

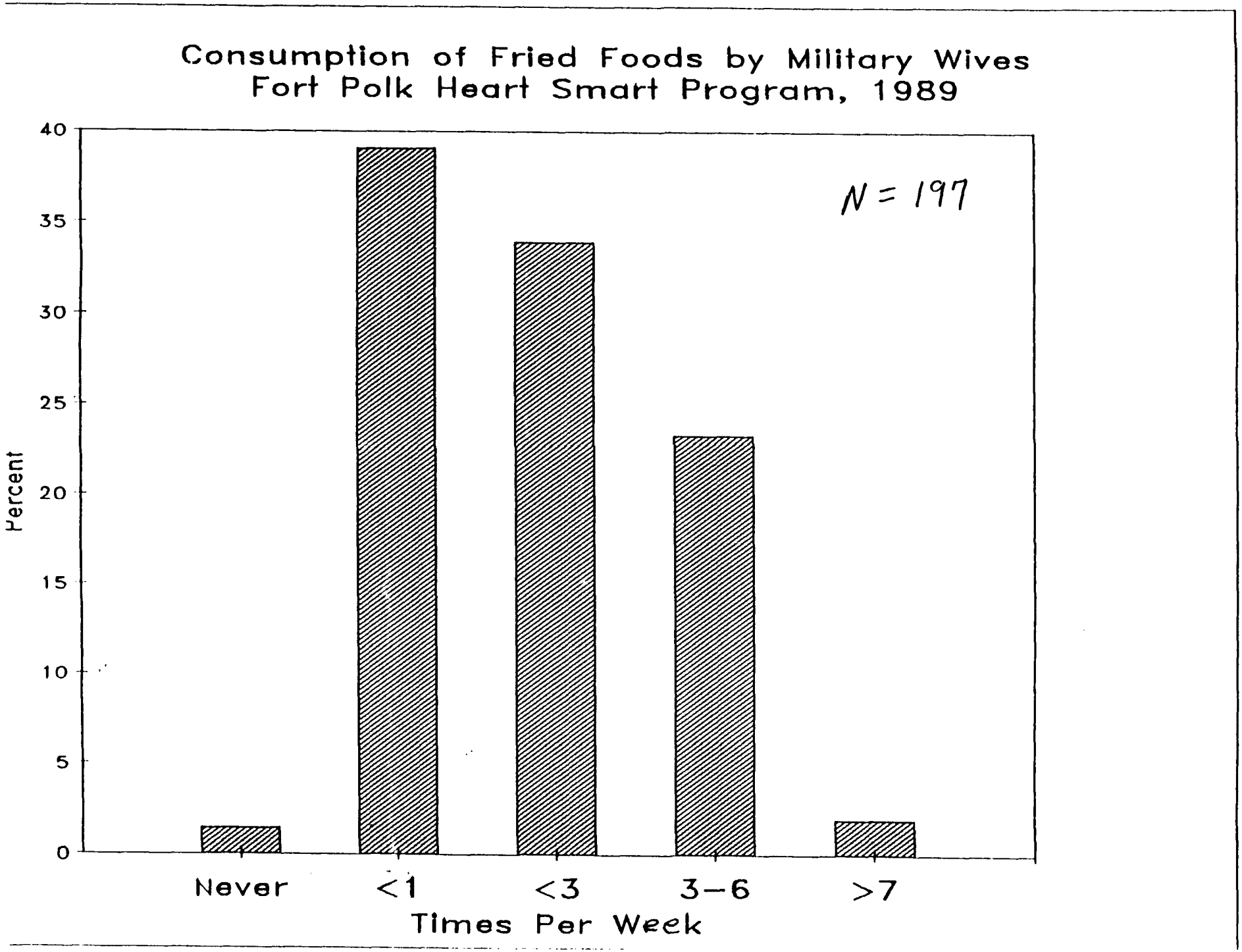


Monthly Expenses for Restaurant Dining
Fort Polk Heart Smart Program, 1989



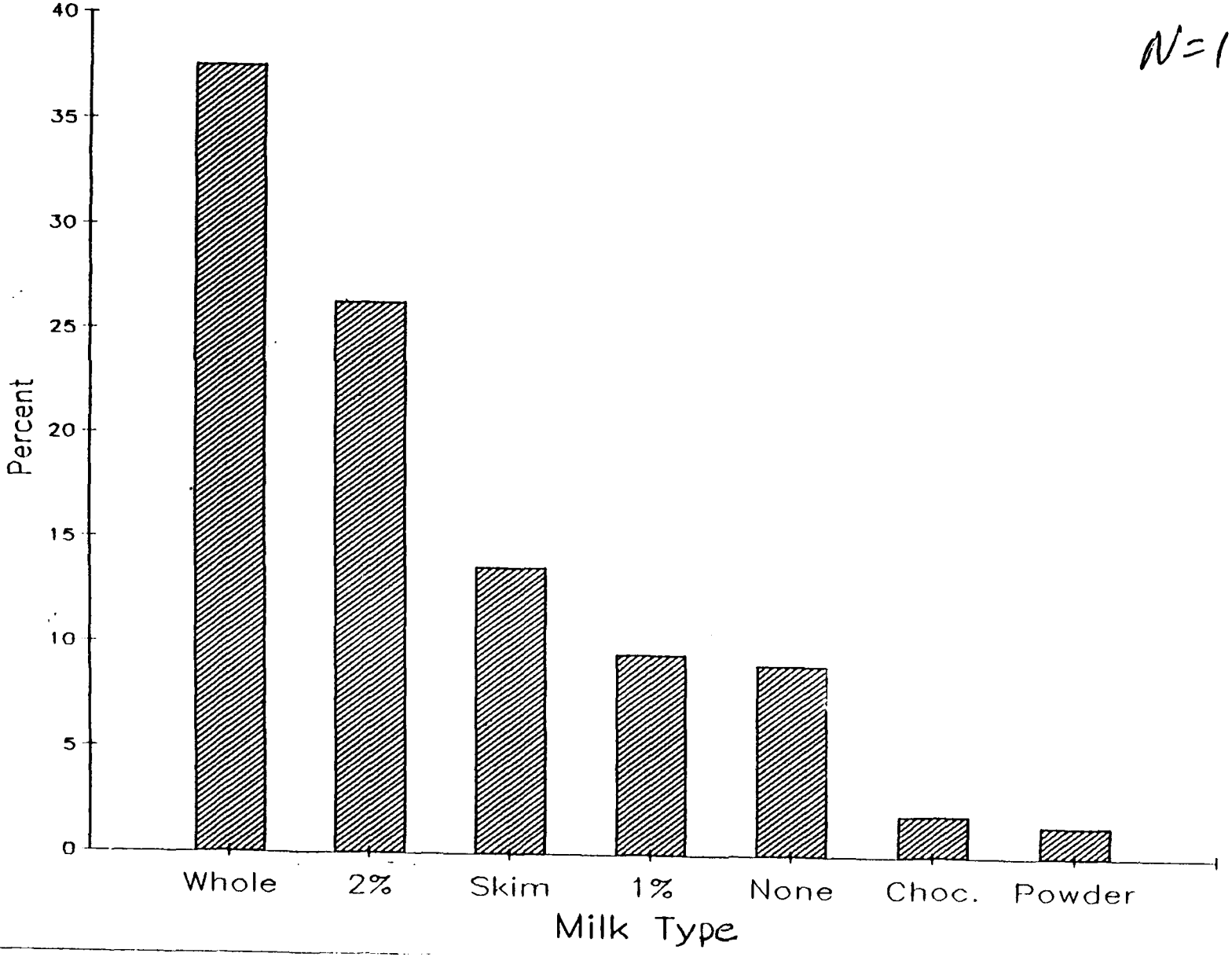
TV Dinner Consumption by Spouses
Fort Polk Heart Smart Project, 1989

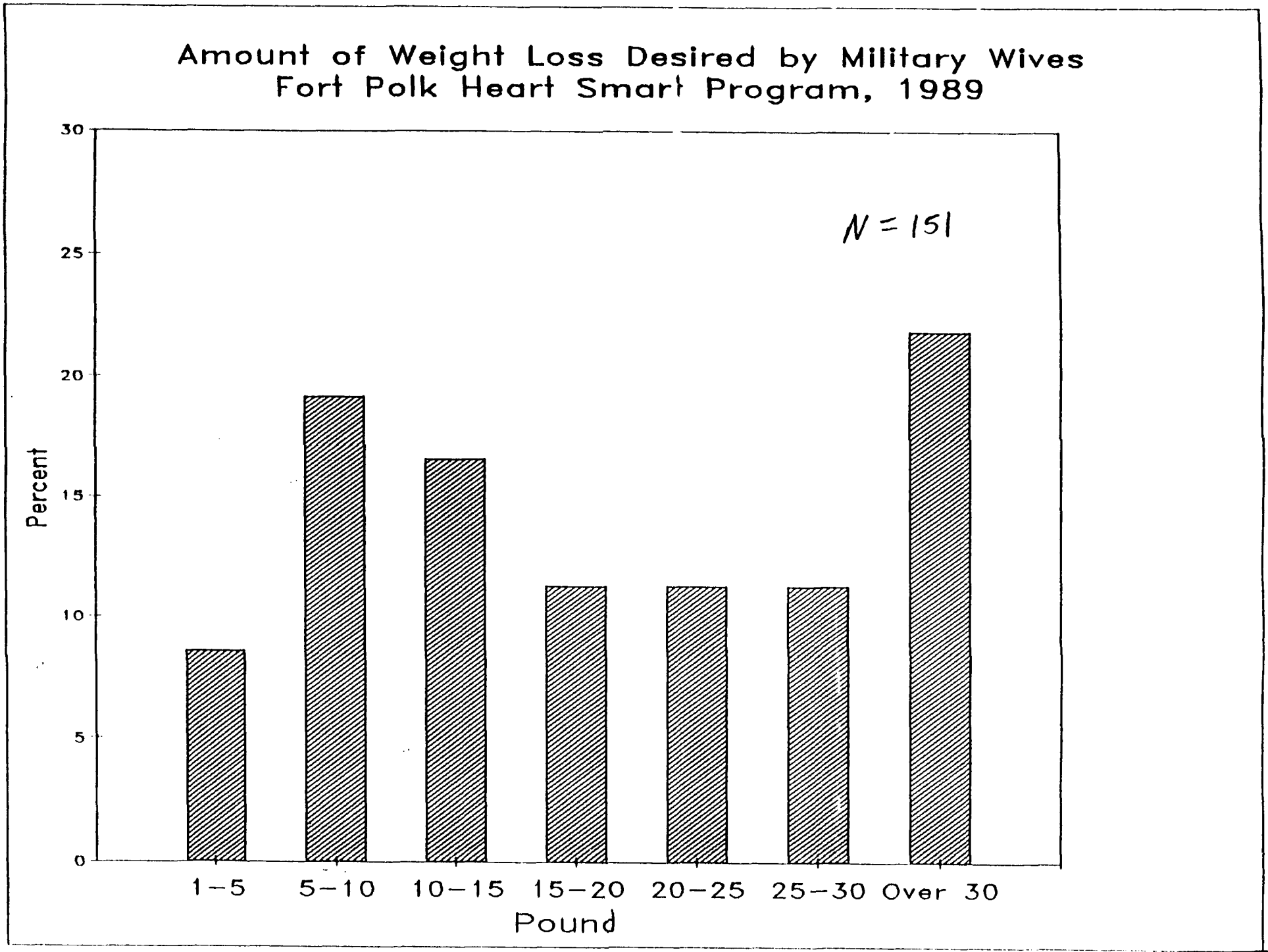



Consumption of Fried Foods by Military Wives
Fort Polk Heart Smart Program, 1989
N = 197
Percent
40
35
30
25
20
15
10
5
0
Never
<1
<3
3–6
>7
Times Per Week

Milk Type Usually Consumed By Spouses Fort Polk Heart Smart Project, 1989

N=197




Amount of Weight Loss Desired by Military Wives
Fort Polk Heart Smart Program, 1989
N = 151
Percent
30
25
20
15
10
5
0
1–5
5–10
10–15
15–20
20–25
25–30
Over 30
Pound

Nutrient Intake by Age

Dietary Component	Age Group 19-24 (n=51)	25-45 (n=135)
Calories, kcal	2015	1812
Protein	71.3	63.5
Animal	50.1	41.6
Vegetable	18.5	17.7
Fat	78.6	72.0
SFA	27.0	26.0
PUFA	14.6	14.6
MONO	27.9	24.3
Carbohydrate	269.7	233.3
Starch	150.7	86.4
Sugar	128.2	114.5
Sucrose	79.4	71.3
Fiber	3.14	2.84
Cholesterol	229	243

Percent Contribution of Macronutrients to Energy Intake by Age

Dietary Component	Age Group 19-24	25-45
Calories	2015	1812
% Protein	14.8	14.2
% Fat	34.2	34.9
% SFA	11.9	12.9
% CHO	53.9	52.4
% Alcohol	0.14	0.47

Electrolyte and Iron Intake by Age

Dietary Component	Age Group 19-24	25-45
Sodium	3418	3111
Potassium	2352	2170
Magnesium	222	222
Calcium	933	803
Phosphorus	1250	1070
Iron	.016	.022

Nutrient Intake by Ethnic Origin

Dietary Component	Ethnicity				
	White (n=125)	Black (n=34)	Hispanic (n=20)	Asian (n=3)	Other (n=4)
Calories, kcal	1906	1771	1754	2018	1946
Protein	66.1	59.6	63.7	104.6	81.3
Animal	43.1	39.7	47.8	77.2	64.3
Vegetable	18.3	17.5	15.0	26.2	15.9
Fat	75.2	68.1	70.4	68.2	101.2
SFA	26.9	24.1	24.2	29.4	32.8
PUFA	15.2	13.0	14.3	5.6	18.6
MONO	25.4	24.3	23.6	24.8	40.0
Carbohydrate	249.4	239.4	219.6	251.3	196.3
Starch	103.9	132.7	63.0	111.4	65.1
Sugar	122.8	113.9	109.8	98.8	69.9
Sucrose	75.5	78.9	60.8	50.1	46.0
Fiber	3.06	2.35	2.27	7.44	3.23
Cholesterol	216	235	301	610	431

Percent Contribution of Macronutrients to Energy Intake by Ethnic Origin

Dietary Component	Ethnicity				
	White	Black	Hispanic	Asian	Other
Calories, kcal	1906	1771	1754	2018	1946
% Protein	14.2	13.4	15.0	20.5	16.5
% Fat	34.6	33.6	36.3	30.2	43.1
% SFA	12.9	11.4	12.6	13.0	13.6
% CHO	53.0	55.1	49.2	50.3	45.1
% Alcohol	0.43	0.19	0.51	0.0	0.0

Electrolyte and Iron Intake by Ethnic Origin

	Ethnicity				
Dietary Component	White	Black	Hispanic	Asian	Other
Sodium	3303	2863	2498	6865	3390
Potassium	2375	1728	1724	4103	2597
Magnesium	238	181	162	415	235
Calcium	914	667	686	1123	506
Phosphorus	1172	979	990	1445	1095
Iron	.024	.014	.010	.022	.011

Age Range and Median of Military Dependents by Husband's Rank

Husband's Rank	(N)	Age Range (yrs)	Median Age (yrs)
Enlisted	(49)	19-46	23
Enlisted (Sergeant & Higher)	(107)	20-42	28.5
Officers	(29)	23-45	34

Nutrient Intake by Husband's Rank

Dietary Component	Husband's Rank: Enlisted (n=49)	Husband's Rank: Enlisted (Sergeant & Higher) (n=107)	Husband's Rank: Officers (n=29)
Calories, kcal	2101	1768	1870
Protein	69.2	64.5	64.4
Animal	48.5	42.5	42.2
Vegetable	17.9	18.3	16.6
Fat	85.2	68.3	75.4
SFA	29.5	23.3	32.1
PUFA	17.1	13.3	15.2
MONO	29.7	23.6	24.1
Carbohydrate	277.5	230.0	239.6
Starch	118.4	108.1	67.0
Sugar	139.9	107.6	123.6
Sucrose	96.9	65.4	65.8
Fiber	2.74	2.91	3.32
Cholesterol	253	239	199

Percent Contribution of Macronutrients to Energy Intake by Husband's Rank

Dietary Component	Husband's Rank: Enlisted	Husband's Rank: Enlisted (Sergeant & Higher)	Husband's Rank: Officers
Calories	2101	1768	1870
% Protein	13.6	14.8	13.8
% Fat	35.7	34.2	34.3
% SFA	12.7	11.5	16.3
% CHO	52.8	52.8	53.4
% Alcohol	0.47	0.37	0.29

Electrolyte and Iron Intake by Husband's Rank

Dietary Component	Husband's Rank		
	Enlisted	Enlisted (Sergeant & Higher)	Officers
Sodium	3531	3044	3195
Potassium	2167	2227	2303
Magnesium	218	222	232
Calcium	899	797	913
Phosphorus	1189	1095	1104
Iron	.019	.023	.014

Nutrient Intake by Meal Period

Dietary Component	Meal Period			
	Breakfast (n=157)	Lunch (n=162)	Dinner (n=184)	Snacks (n=170)
Calories, kcal	346	529	700	462
Protein	12.3	19.9	30.4	8.6
Animal	7.5	12.8	22.8	4.3
Vegetable	3.7	5.1	6.9	3.9
Fat	12.2	21.5	31.4	15.1
SFA	5.6	7.4	10.8	4.9
PUFA	2.1	4.2	6.1	3.5
MONO	4.0	7.3	11.1	5.0
Carbohydrate	48.0	66.4	75.8	76.5
Starch	32.9	28.1	30.9	23.2
Sugar	22.3	29.9	29.2	48.7
Sucrose	10.8	20.0	18.9	30.9
Fiber	0.39	0.79	1.22	0.76
Cholesterol, mg	72	66	99	26

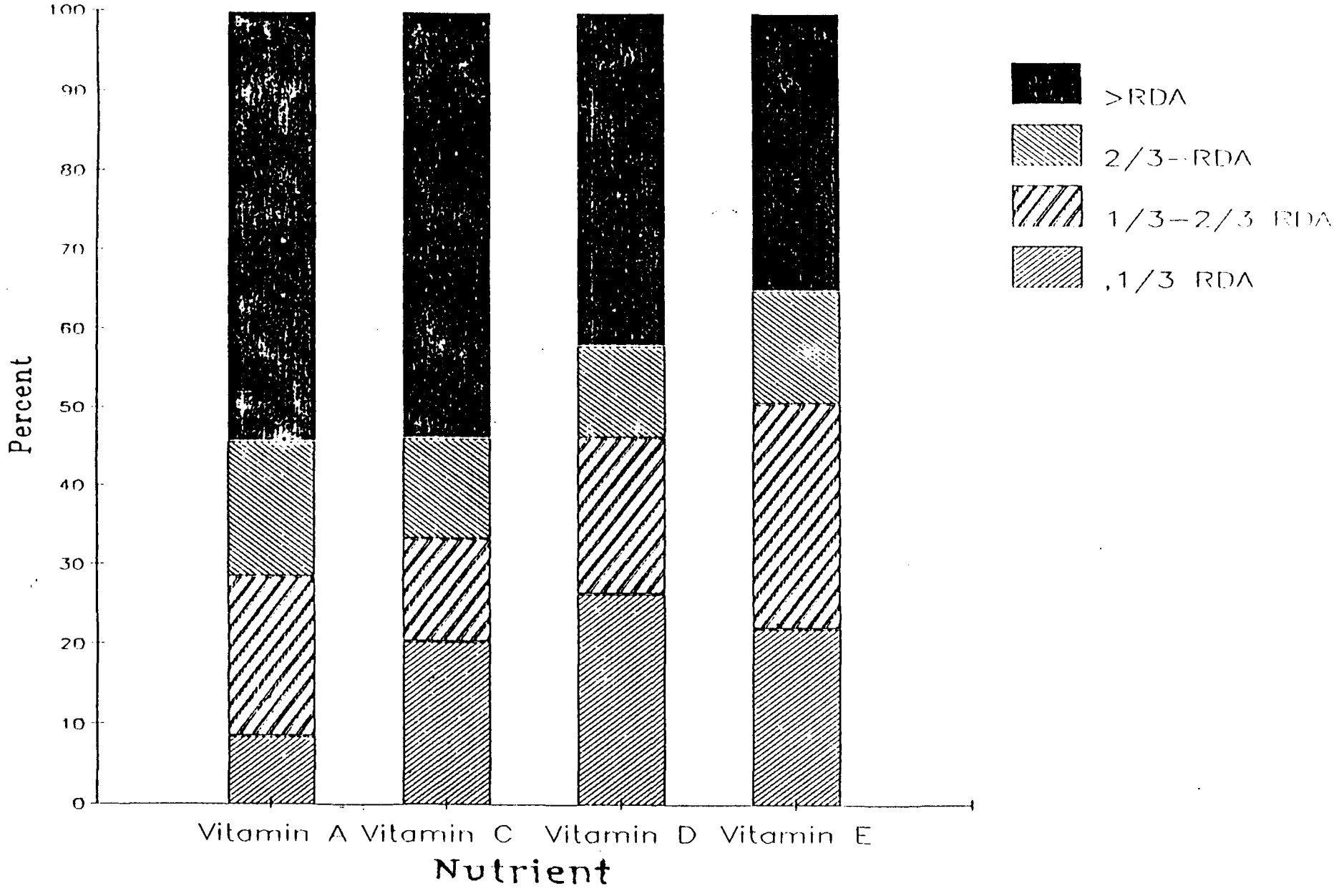
Percent Contribution of Macronutrients to Energy Intake by Meal Period

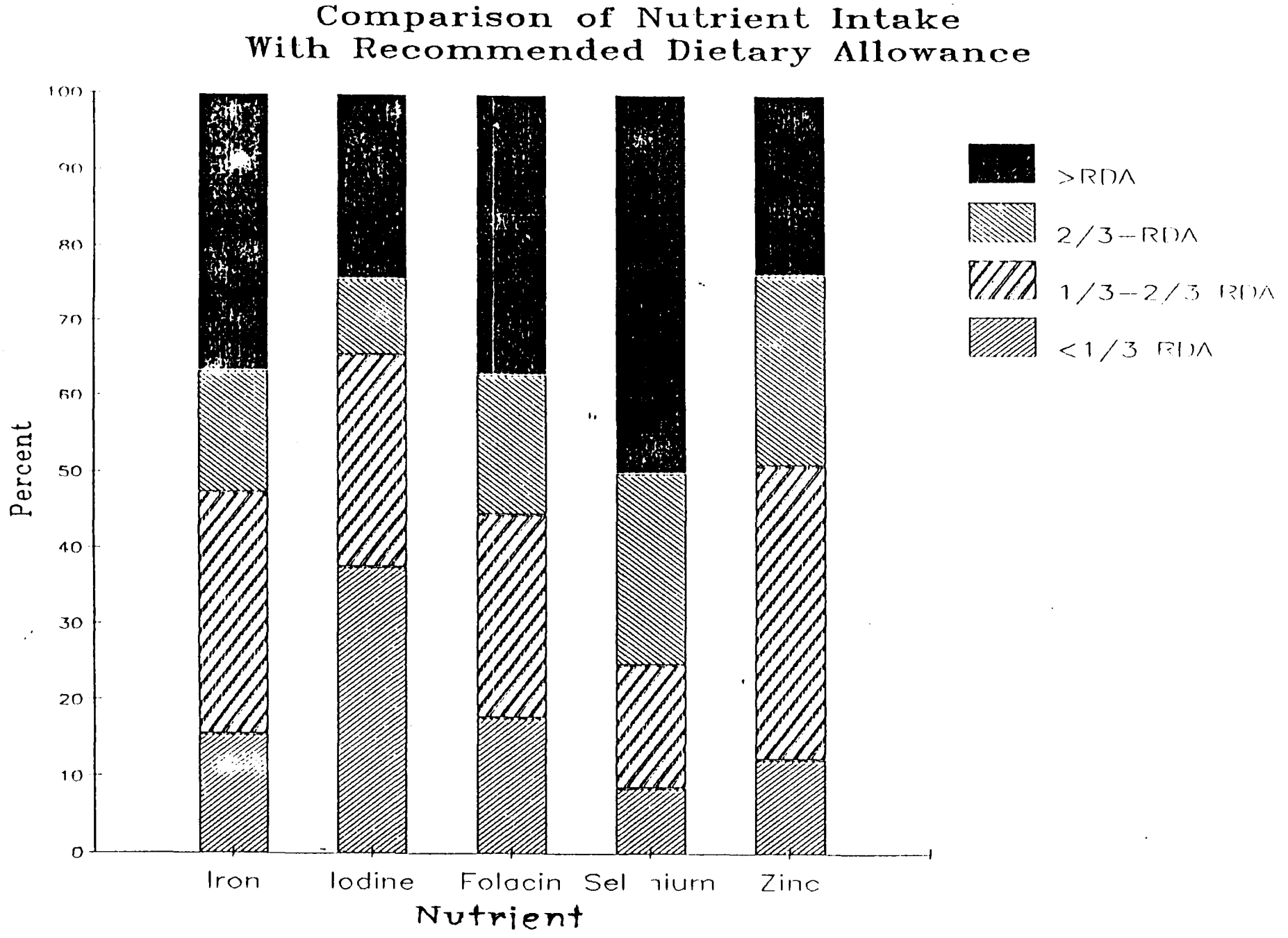
Dietary Component	Meal Period			
	Breakfast	Lunch	Dinner	Snacks
Calories	346	529	700	462
% Protein	14.1	15.2	17.6	7.6
% Fat	28.6	34.9	37.2	26.1
% SFA	22.1	11.8	12.4	9.1
% CHO	58.8	52.0	46.0	69.7
% Alcohol	0.29	0.01	0.49	0.89

Electrolyte and Iron Intake by Meal Period

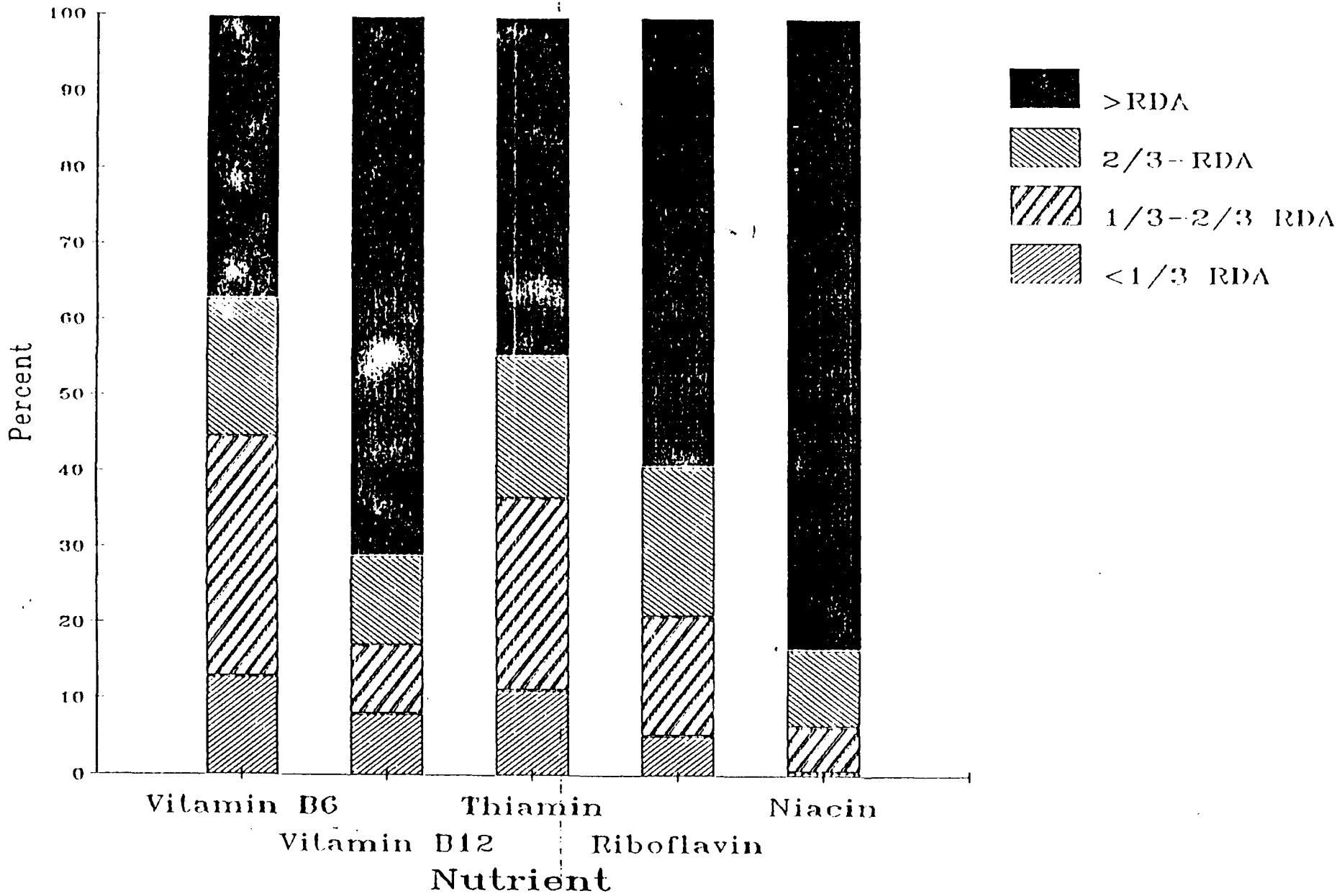
Dietary Component	Meal Period			
	Breakfast	Lunch	Dinner	Snacks
Sodium	506	1161	1432	373
Potassium	533	542	857	492
Magnesium	54	56	79	53
Calcium	261	215	258	192
Phosphorus	273	308	427	218
Iron	.008	.003	.004	.007

Comparison of Nutrient Intake With Recommended Dietary Allowance

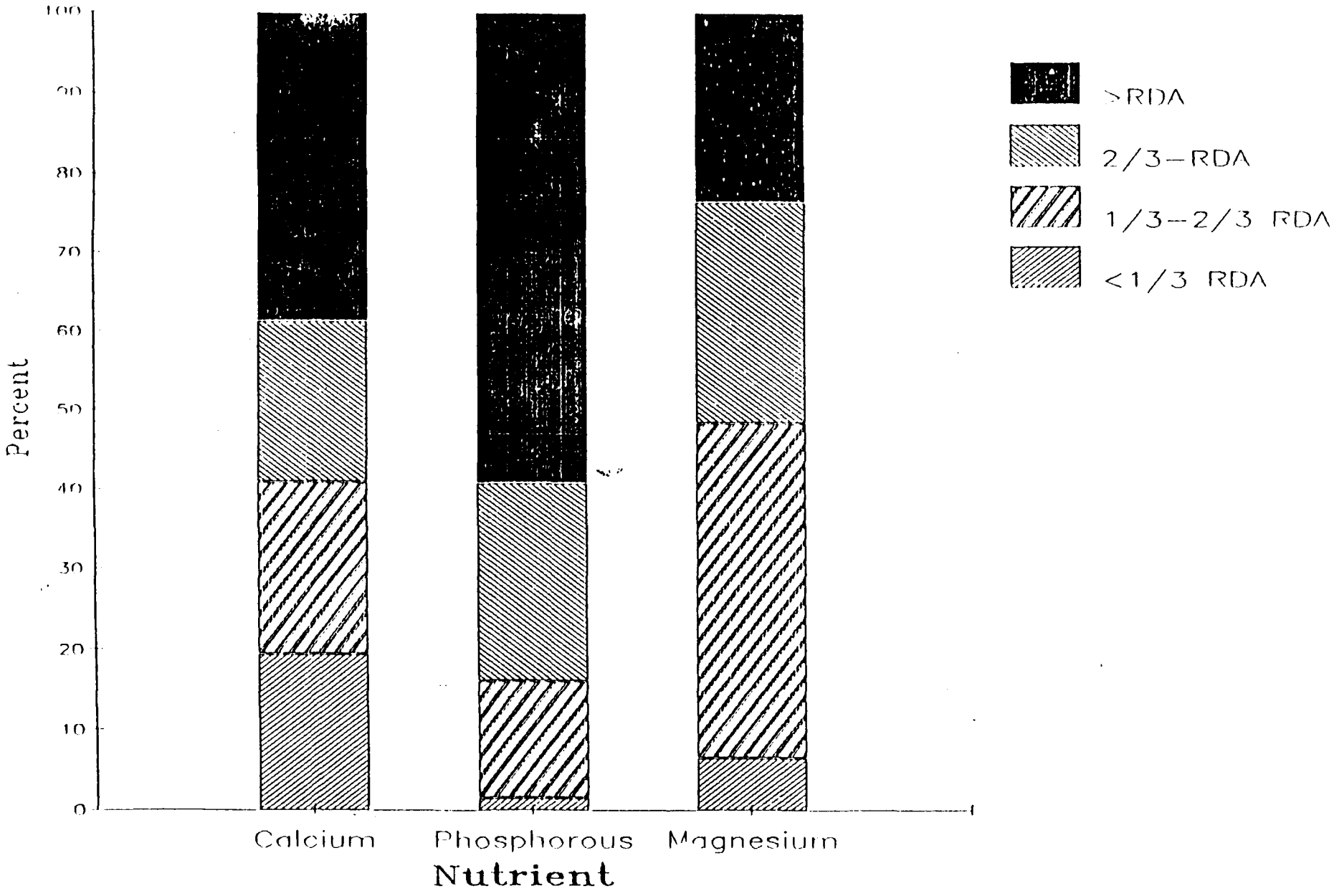



Comparison of Nutrient Intake
With Recommended Dietary Allowance
Percent
100
90
80
70
60
50
40
30
20
10
0
Iron
Iodine
Folacin
Selenium
Zinc
Nutrient
>RDA
2/3–RDA
1/3–2/3 RDA
<1/3 RDA

Comparison of Nutrient Intake With Recommended Dietary Allowance



Comparison of Nutrient Intake With Recommended Dietary Allowance



MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL
COMPARED WITH THREE NATIONAL SURVEYS
(n=188)

Dietary Component (g)	Fort Polk 1989	NHANES II 1976-1980	LRC 1982	CSFII 1985	CSFII 1986
Calories, kcal	1869	1643	1984	1528	1473
Protein	66	64	75	61	60
Fat	74	68	90	63	61
SFA	26	24	34	23	22
PUFA	15		16	13	12
MUFA	25		34	23	22
Carbonyarate	243	187	205	177	169
Starch	104				
Total Sugar	118				
Sucrose	73				
Fiber	2.9				
Cholesterol, mg	239	278	332	280	272

NHANES II - Second National Health and Nutrition Examination Survey - Women 25-34 years.

LRC - Lipid Research Clinics - White women - 25-29 yrs.

CSFII - Women 19-50 yrs. - Nationwide Food Consumption Survey - Continuing Survey of Food Intakes by Individuals (an average of 4 days).

MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL
COMPARED WITH THREE NATIONAL SURVEYS
(n=188)

Dietary Component	Fort Polk 1989	NHANES II 1976-1980	LRC 1982	CSFII 1985	CSFII 1986
Calories, kcal	1869	1643	1984	1528	1473
Protein, % kcal	14.3	--	15.4	16.2	16.7
Carbohydrate, %kcal	52.7	--	41.9	46.4	45.9
Fat, %kcal	34.7	36.3	39.9	36.8	36.7
Alcohol, %kcal	0.4	--	--	--	--

NHANES II - Second National Health and Nutrition Examination Survey - Women 19-50 yrs.

LRC - Lipid Research Clinics - White women - 25-29 yrs.

CSFII - Women 19-50 yrs. - Nationwide Food Consumption Survey - Continuing Survey of Food Intakes by Individuals (an average of 4 days).

MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL
COMPARED WITH THREE NATIONAL SURVEYS
(n=188)

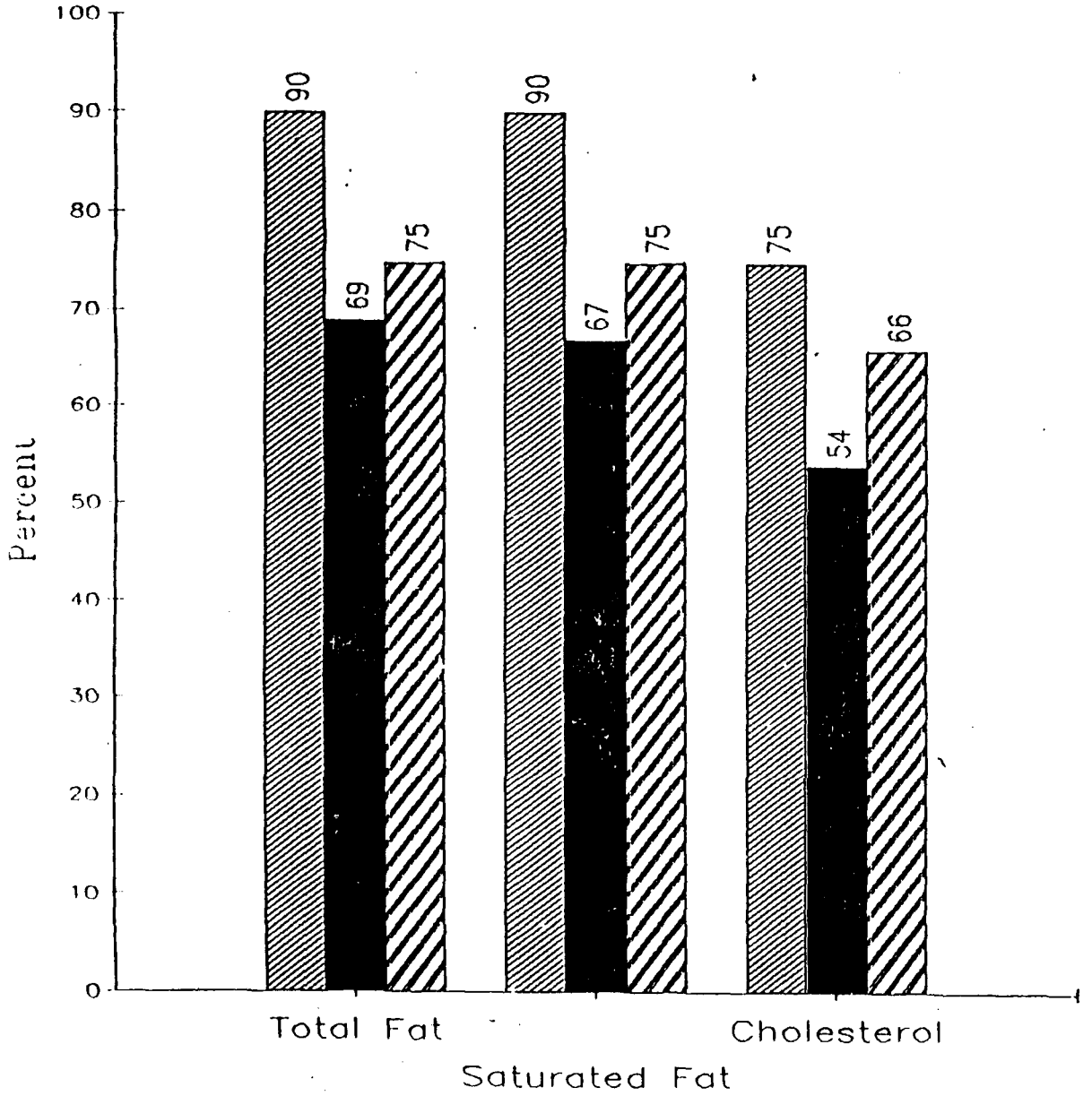
Dietary Component (g)	Fort Polk 1989	NHANES II 1976-1980	LRC 1982	CSFII 1985	CSFII 1986
Calories, kcal	1869	1643	1984	1528	1473
Protein	66	64	75	61	60
Fat	74	68	90	63	61
SFA	26	24	34	23	22
PUFA	15		16	13	12
MUFA	25		34	23	22
Carbohydrate	243	187	205	177	169
Starch	104				
Total Sugar	118				
Sucrose	73				
Fiber	2.9				
Cholesterol, mg	239	278	332	280	272

NHANES II - Second National Health and Nutrition Examination Survey - Women 25-34 years.

LRC - Lipid Research Clinics - White women - 25-29 yrs.

CSFII - Women 19-50 yrs. - Nationwide Food Consumption Survey - Continuing Survey of Food Intakes by Individuals (an average of 4 days).

Comparison of Army Wives vs. 2 National Health Surveys
Percent Not Meeting AHA Dietary Recommendations



LEGEND

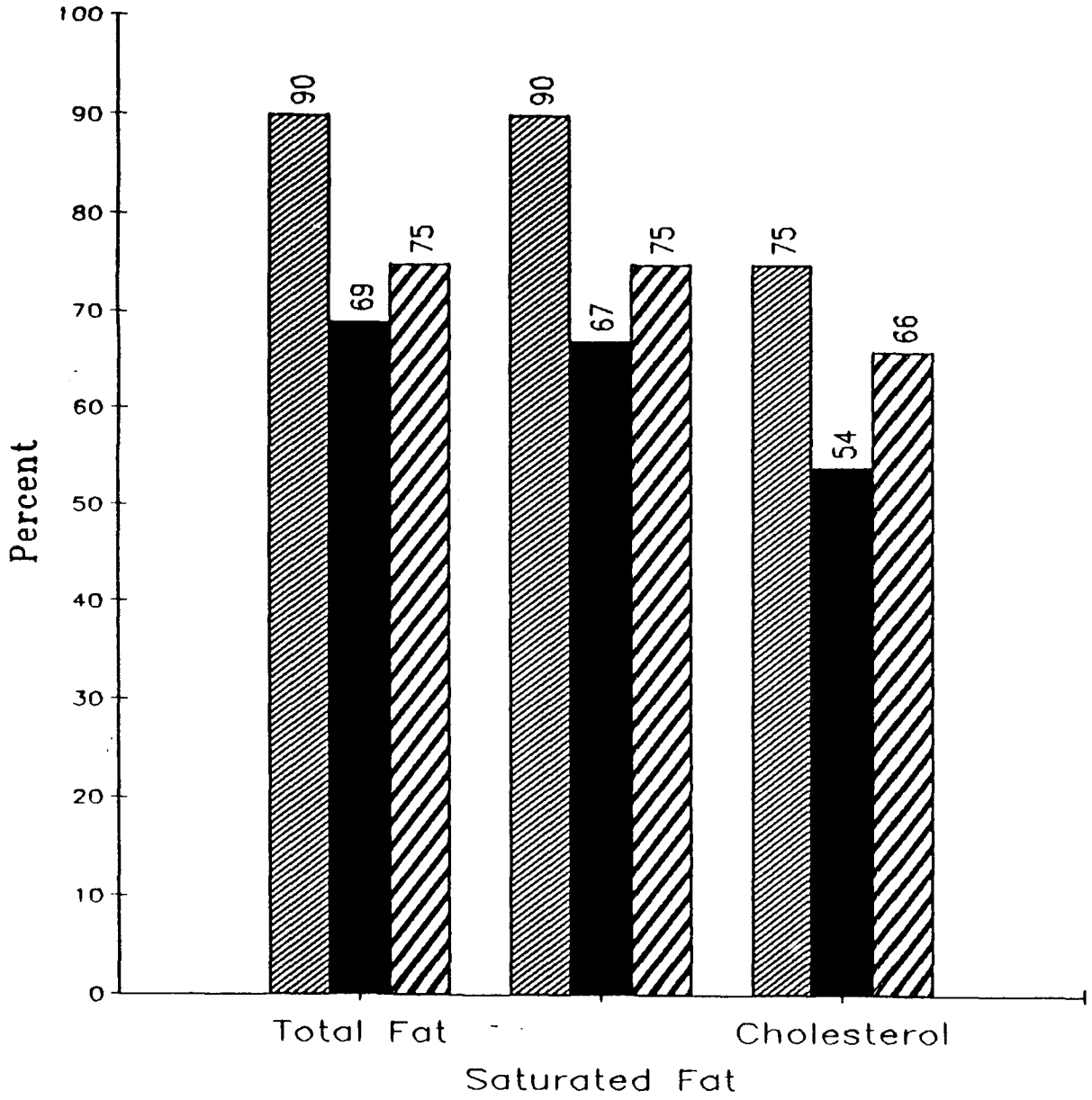
- Lipid Research Clinic
- Fort Polk
- CSFII

Total Fat > 30% kcal

Saturated Fat > 10% kcal

Cholesterol > 100mg/1000 kcal

Comparison of Army Wives vs. 2 National Health Surveys
Percent Not Meeting AHA Dietary Recommendations



LEGEND

Lipid Research Clinic

Fort Polk

CSFII

Total Fat > 30% kcal

Saturated Fat > 10% kcal

Cholesterol > 100mg/1000 kcal

MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL
COMPARED WITH TWO NATIONAL SURVEYS
(n=188)

Dietary Component (mg)	Fort Polk	NHANES II	CSFII	
	1982	1976-1980	1985	1986
Sodium, g	3.2	2.4	2.4	2.3
Potassium, g	2.2	2.1	2.1	2.1
Calcium	834	636	614	621
Phosphorus	1118	1021	966	958
Magnesium	221	--	207	205
Iron	20	11	10	10

CSFII - Women 19-50 yrs. - Nationwide Food Consumption Survey - Continuing Survey of Food Intakes by Individuals (an average of 4 days).

NHANES II - Second National Health and Nutrition Examination Survey - Women 25-34 years.